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ESTROGEN PRODUCTION BY THE TESTIS 579
Morgan Berthrong, Willard E. Goodwin and William Wallace Scott

METABOLIC ACTIONS AND FATE OF INTRAVENOUSLY ADMINISTERED ADRENOCORTICOTROPIC HORMONE IN MAN 593
George Sayers, Thomas W. Burns, Frank H. Tyler, B. V. Jager, Theodore B. Schwartz, Emil L. Smith, L. T. Samuels and Horace W. Davenport

THE RENAL CLEARANCE OF CHORIONIC GONADOTROPIC HORMONE IN PREGNANCY, IN NEOPLASM OF THE TESTIS AND IN HYDATIDIFORM MOLE 615
Clifford F. Gastineau, A. Albert and Lawrence M. Randall

COARCTATION OF THE AORTA ASSOCIATED WITH ABNORMAL DIGITS, OVARIAN INSUFFICIENCY, AND SHORTNESS OF STATURE 622
Melvin L. Goldman, Henry A. Schroeder and Palmer H. Futcher

OVARIAN GRANULOSA CELL TUMOR AND ACROMEGALY 630
Harold Speert

ESTIMATION OF URINARY GONADOTROPIN OF THE NONPREGNANT HUMAN BY THE MOUSE UTERINE WEIGHT AND OVARIAN HYPEREMIA RESPONSES 636
Charles W. Lloyd, Muriel Morley, Kathryn Morrow, Julia Lobotsky and Edward C. Hughes

THE EXCRETION OF NEUTRAL LIPID-SOLUBLE REDUCING SUBSTANCES BY INFANTS 646
Charles F. Matson and Bernard B. Longwell

LETTER TO THE EDITOR: HYPERSENSITIVITY TO PITRESSIN 650
John Brice Plass

ABSTRACTS OF PAPERS PRESENTED AT THE THIRTY-FIRST ANNUAL MEETING OF THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS 651

RECIPIENTS OF THE 1949 AWARDS OF THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS 685

RECIPIENT OF THE 1949 VAN METER PRIZE AWARD OF THE AMERICAN GOITER ASSOCIATION 687

FELLOWSHIPS FOR LATIN-AMERICAN PHYSICIANS 689

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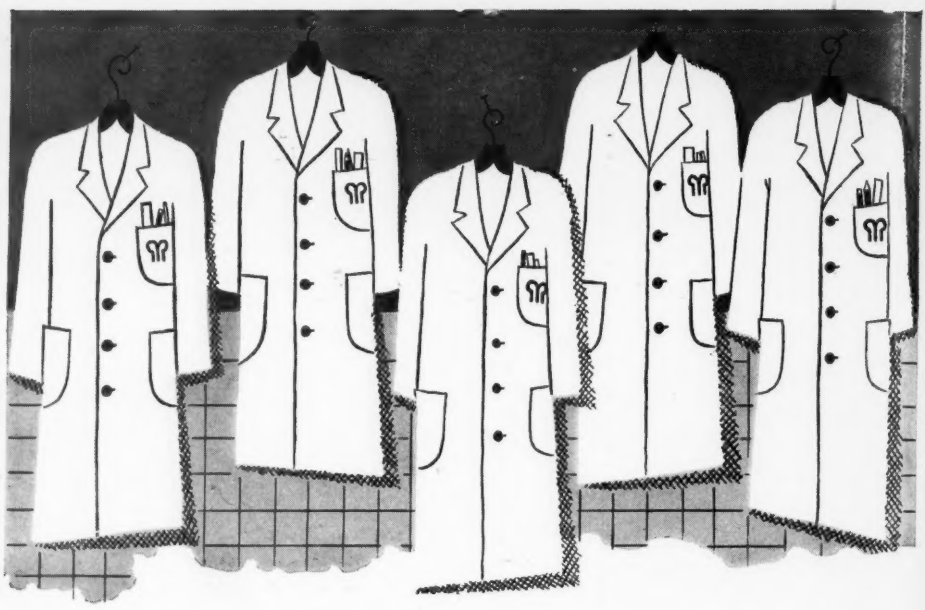

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ESTROGEN PRODUCTION BY THE TESTIS*†

MORGAN BERTHRONG, M.D., WILLARD E. GOODWIN, M.D.‡ AND WILLIAM WALLACE SCOTT, M.D.

From the Department of Pathology and the James Buchanan Brady Urological Institute, Johns Hopkins Hospital, Baltimore, Maryland

ONE hundred years ago Berthold (1), on the basis of transplantation experiments in the cock, presented evidence that the testes exert decided effects on the organism through the blood stream, independent of established nervous pathways. Since then tremendous energy has been expended on determining the nature of the hormones elaborated by the testis, the cells responsible for their production and the physiologic actions of the hormones produced. Primarily, workers have concerned themselves with a study of testicular substances having masculinizing effects but considerable work has been done in regard to feminizing testicular influences. Without reviewing the evidence, it seems fair to say that most workers agree that masculinizing effects are secondary to the production of an androgenic substance or substances presumed to come exclusively from the interstitial cells of Leydig independent of the generative tissue of the testis (2, 3). However, there is a considerable body of evidence to suggest that tubular activity contributes to hormone production.

Fellner in 1921 (4) appears to have been the first to suggest, on the basis of experimental findings, that the testis produced a substance with a feminizing or estrogenic action, observing an increase in uterine weight

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of the castrate guinea pig after injection of a lipoid extract of bull testis. With suitable stains, lipids were found by him to predominate normally in the Leydig and Sertoli cells. Since the work of Fellner others have confirmed the presence of substances with estrogenic activity in testicular extracts. Brouha and Simonnet (5), Zondek (6), Dorfman, Gallagher and Koch (7) and Cunningham and his associates (8) have clearly demonstrated the presence of estrogenic substance in bull testis; Zondek, the large quantity of estrogen in stallion testis; and Laqueur and deJongh (9), the presence of estrogen in human testis. In 1940 Beall (10) isolated crystalline alpha-estradiol and estrone from horse testis.

These studies are few in comparison to the number of studies made of male urine to determine the presence or absence of estrogens. In this regard estrogens have been found in the urine of normal men, stallions, geldings and colts, as well as other male animals. In 1942 one of us with Vermeulen (11) found moderate amounts of estrogen in the urine of aging men with prostatic cancer. Generally, following castration, urinary estrogens fell to low levels; but 3 individuals showed single high postcastrational values.

That estrogen is produced by the testis seems likely from the foregoing reports of the presence of estrogen in testicular extracts and the decrease in urinary estrogens following castration. Where in the testis is estrogen produced?

With increasing time, evidence accumulates indicating that estrogen is produced by the sustentacular cells of Sertoli. It appears difficult to establish priority for this concept, but certainly Zuckerman and McKeown (12) were aware of this possibility. These authors in a study of 243 dogs found testicular tumors present in 35 of them. Fifteen of these tumors were classified as adenocarcinomata, and in 5 of these Sertoli elements predominated. These authors, aware of the concept of Witschi (13)—that the testicular sustentacular cells of Sertoli are homologous with the granulosa cells of the ovarian follicle, both arising from the follicle cells of the primordial gonia—suggested that the estrogenic potency of these tumors was related to the fact that Sertoli cells take part in their formation. To them, estrogenic potency or action was evidenced in these dogs with testis tumors by squamous metaplasia of the prostatic epithelium, enlargement of the nipples, increased pigmentation of the skin over the abdomen and stratification of the urethral epithelium, all of which occurred in control dogs injected with estrogenic substances.

In reviewing the literature Zuckerman and McKeown were impressed with the great similarity of the tumors producing feminizing changes in their dogs to the three testicular tumors in dogs reported by Greulich and Burford (14). The writers in reviewing these articles were impressed with

the striking similarity in histologic pattern of the adenocarcinomata reported by the above authors and the "Sertoli-cell tumor" to be described here.

In 1945 Huggins and Moulder (15) in a study of 5 dogs with feminizing testicular tumors of spontaneous origin reported the results of their investigations which included a description of the gross and microscopic anatomic status of the animal, an analysis of the tumor lipids and of the tumor estrogens. Their results clearly indicate that in the dog, feminizing tumors exist which are high in lipid content, high in estrogen content, and give the appearance of being composed predominantly of Sertoli cells which retain their normal character of accepting fat stains. They concluded that "the cells of Sertoli in the testicular tubules produce estrogen."

Recently one of us, having aided in the estrogen assays of the animals reported by Huggins, had occasion to recall his work and to institute a study of a male dog with a feminizing testicular tumor.

Case History of Dog T

This male English setter had always been well until 12 years of age. At this time it was first noticed that the hair became generally somewhat sparse and that over his lower back it was occasionally lost entirely. Local veterinarians felt that it did not represent the usual picture of "mange," but were unable to make other diagnoses. This complete loss of hair over the base of the tail occurred at intervals, with periods of regrowth, for no apparent cause and with no therapy. This area apparently was extremely itchy. About the same time, marked pigmentation of the skin of the abdomen and testes was noted, to which no significance was attributed. The pigmentation persisted until death, with what was definitely thought to be alternate increases and decreases of intensity. Six months after the onset of the above signs, it was noticed that male dogs of the neighborhood were being attracted to the house. It then also became obvious that these male dogs were being sexually attracted to Dog T and repeatedly attempted to mount him, whenever he was taken out. Dog T vigorously resisted such approaches. The dog's health remained good however, until one year after the onset of the skin pigmentation when it was noticed that the right testis was greatly enlarged to at least twice the size of the left. The right testis was hard and nontender. At this time slight difficulty with urination seemed apparent, but this symptom was not definite. There was also noticed marked enlargement of the nipples, particularly of the posterior ones. There was no weight loss, anorexia or apparent discomfort. Because it seemed that all the above findings must be related, Dog T was brought to the hospital for study.

Physical and laboratory findings

Examination of this animal revealed no evidence of weight loss and good general condition except for moderate alopecia, particularly of the abdomen and tail. There were numerous spots of black pigment in the skin of the abdomen and scrotum. The breasts were enlarged (Fig. 1). The right testis was stony hard and twice the size of the left. The prostate gland was enlarged to 2 or 3 times normal size.

The dog was placed in a metabolism cage and urine collected for a period of five days, 3800 cubic centimeters being obtained during this period. Analysis showed that this

animal excreted 1.6 milligrams of 17-ketosteroids per day and 20 international units of estrogen per day. Follicle-stimulating hormone determination gave negative results in all dilutions, indicating less than 38 mouse units per day. Simply expressed: urinary estrogens were high, 17-ketosteroids low and gonadotropins not demonstrated.

Decision was made to remove the testicular tumor and to take a specimen of the prostate gland and one of the breasts for biopsy. This was begun, but unfortunately the animal died postoperatively, presumably from too much nembutal, and all tissues necessary for study were obtained immediately postmortem.

FIG. 1. Photograph of abdomen of Dog T, with the pigmented skin, prominently enlarged nipples and enlarged scrotal sac plainly demonstrated.

Autopsy

The right testis was found almost completely replaced by a tumor, $4.5 \times 4.5 \times 6$ centimeters and weighing 45 grams (Fig. 2). The epididymis was normal. Cross sections of the tumor showed hard, moist, greyish-yellow tissue in which softer, very grey tumor tissue $4 \times 3 \times 6$ centimeters, containing sanguineous fluid, surrounded the abdominal aorta. Examination of the left testis and of all other tissues save of the central nervous system, revealed nothing abnormal.

Microscopic examination

"Sections of the tumor show atropic testis tubules compressed by a nonencapsulated tumor (Plate 1: Figs. 3 and 4). There is a tubular arrangement in some areas although definite lumina are seldom present. The appearance is occasionally that of solid testicular tubules, but most regions show large nodules and sheets of cells (Fig. 5). The tumor cells often present a palisading effect with their long axes perpendicular to the basement membranes of the tubules or to connective tissue strands and blood vessels of the solid portions. In some areas, the tumor is scattered through atrophic testis, and here the appearance is that of either local infiltration inside intact testis tubules or of the origin

FIG. 2. Hemisection of the tumor. The tumor bulges from the cut surface. It is arranged in lobules with areas of hemorrhage and softening. It is grossly circumscribed, markedly compressing the surrounding testicular tissue.

of the tumor within the tubules themselves (Fig. 6). The nuclei of the tumor are large and vesicular with prominent nucleoli. Mitotic figures are rare. The cytoplasm is pale, scanty and forms, in some areas, a syncytium. Fat stains show abundant fat droplets, large and small, in the tumor cells as well as in the connective tissue immediately surrounding the smaller tubules (Fig. 7). Leydig cells are not seen.

"The tumor in the periaortic nodes is composed of large sheets of similar cells which contain much less lipoid.

"The prostate reveals striking squamous metaplasia (Fig. 8). The breasts show marked cystic hyperplasia, giving a 'thyroid' appearance to the low power view (Fig. 9). The left testicle shows atrophy with but few Leydig cells. Other sections are normal."

PLATE 1

Hematoxylin and eosin stained sections of the testicular tumor (Zenker-formol fixation).

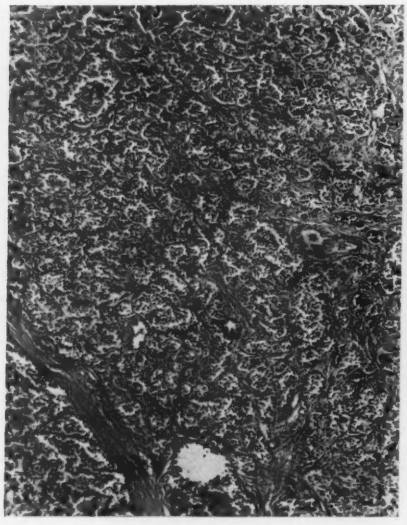

FIG. 3

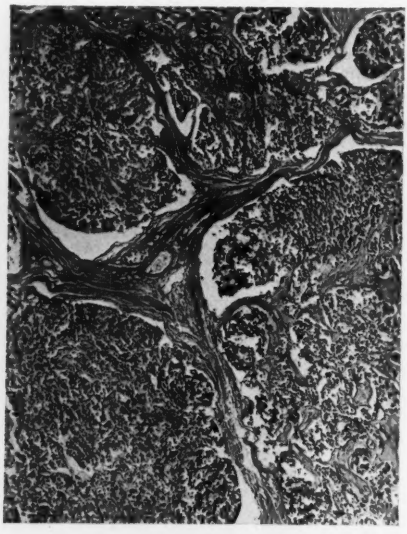

FIG. 4

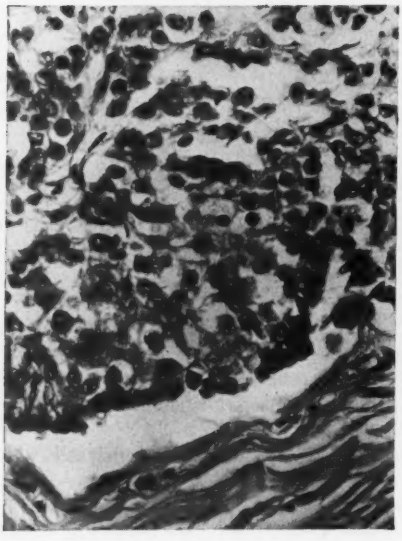

FIG. 5

FIG. 6

Analysis of the tumor for estrogen gave a value of 7.2 micrograms per kilogram expressed in terms of alpha-estradiol, a value between those reported by Huggins for these tumors.

In summary, a sudanophilic tumor with a high content of estrogen is seen to bear resemblance to those described in dogs as adenocarcinomata, "tubular adenomas" and as "Sertoli cell tumors." Squamous metaplasia of the prostate epithelium and cystic hyperplasia of the breast, similar to that found in dogs experimentally given estrogen is present. This tumor, described as benign in dogs, has here followed a prolonged course but has been found to have metastasized to abdominal lymph nodes.

DISCUSSION

Let us now turn to the human for evidence of estrogen production by the testis. In 1942 Witschi and Mengert in a study of two "sisters," examples of genetic intersex, presented evidence supporting their contention that "the sustentacular cells of the seminal tubules were the producers of female sex hormones which predominated in the hermaphrodites (the two "sisters") prior to castration." These two "sisters" observed by Witschi were almost identical in anatomic status. They were of almost equal age, 24 and 26 years, respectively. They had been raised as girls, possessed feminine voices and orientation, had well-developed breasts and a straight-topped female escutcheon of pubic hair. Their skin was smooth, and facial skin free of beard. However, their sex organs were predominantly male in character. Each had a bifid scrotum with two well-developed testes which on biopsy showed the tubular appearance of testis. Before surgery, urinary hormonal studies made in one of them showed moderate to normal estrogenic activity and only slight androgenic and gonadotropic activity. Three and one-half years after castration, urinary estrogen had decreased markedly and gonadotropic activity had increased to 100 rat units per day. At the time of removal of the scrotal gonads, exploratory laparotomy revealed no female pelvic organs. Sections of the scrotal gonads were said to show that the seminal tubules contained "an abundance of sustentacular cells of Sertoli." Maturation of sperm, to and beyond the stage of primary spermatocytes, appeared only rarely. No evidence of ovarian tissue could be seen.

FIG. 3. (×60) The low power photomicrograph shows a suggestively tubular pattern. In spite of prompt fixation and slow dehydration, considerable shrinkage unfortunately occurred.

FIG. 4. (×60) Other areas of the primary tumor and most of the metastatic tumor show more solid sheets of cells separated by acellular connective tissue strands.

FIG. 5. (×500) Cellular detail of the more solid portions of the tumor.

FIG. 6. (×300) Atrophic testicular tubules, adjacent to the tumor, surround a tubule replaced by tumor cells although the basement membrane remains intact. This probably represents invasion from the main tumor mass although multicentric origin of such tumors has been postulated on the basis of such a finding.

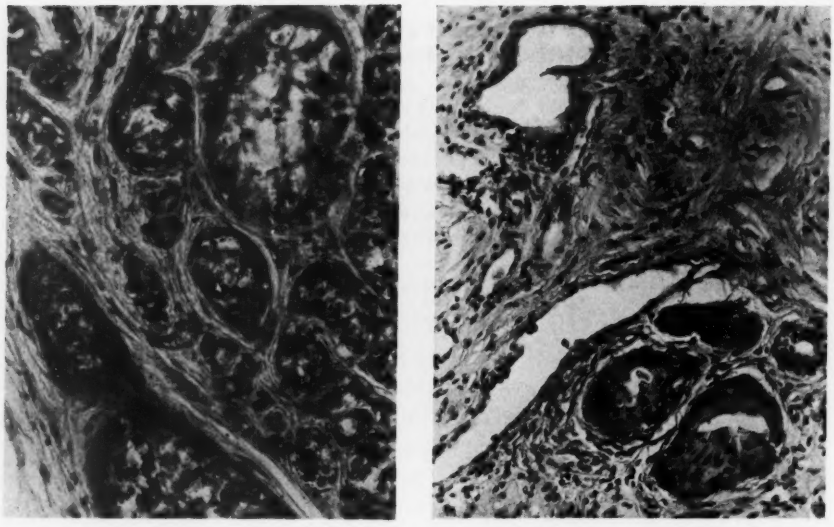

FIG. 7. (×300) Sudan stained frozen section of the main tumor mass. There is considerable fat in the tumor cells as well as in the connective tissue immediately surrounding the tumor masses, as seen in low center of figure.

FIG. 8. (×300) Hematoxylin and eosin stained section of prostate shows clearly the squamous metaplasia seen throughout all sections of the prostate.

FIG. 9. (×60) Hematoxylin and eosin stained section through breast tissue in one of the enlarged nipples shows extreme dilatation of breast acini and ducts, surrounding a longitudinally cut mammary duct.

Estrogen-producing testicular tumors occurring in dogs, such as in our case, Huggins' and others, must however, be excessively rare in humans. This is particularly true if masculine pseudohermaphrodite patients be excluded. Similar tumors without endocrine disturbances are also rarely reported.

In 1905, Pick (16) described both single and multiple adenomas in ectopic testes of masculine pseudohermaphrodites. These were called "adenoma tubulare testicularii ovarii" because of their similarity to ovarian tumors in both 'normal' women with menstrual difficulties, and in women with associated masculinization. This type of tumor has been considered as arising from Sertoli cells, being composed of cylindrical cells with palisading nuclei and cytoplasmic syncytia arranged in a tubular pattern, although other theories have been entertained. Chevassu (17) in 1906 found 3 of 128 testicular tumors to be tubular adenomas of either Sertoli cell or spermatogonia origin. Ewing (18) described 2 "pure adenomas" in cases of undescended testes and another in a pseudohermaphrodite. The microscopic picture described appears similar to that seen in dogs. Monaschkin (19) reported a case of gynecomastia occurring in an otherwise normal male but it is difficult to ascertain from either the pictures or the description whether this tumor could be considered an adenoma of the tubules or whether it was an interstitial cell or adrenal rest tumor. Rea (20) noted a case of a "carcinoma" of the undescended testes of a pseudohermaphrodite and considered that there were 51 cases of pseudohermaphrodites with ectopic testicular tumors, presumably adenomas, reported in the literature.

Engle and associates (21) in reporting a follow-up on a case of true hermaphroditism, who had been made "male," described the development of breasts at puberty even though the female elements had been removed four years before. Their photographs of the sections of the testis biopsy specimen reveal seminiferous tubules packed with Sertoli cells, which look much like the tumor described here.

Pace and Cabot (22) described 3 cases, taken from 24 specimens of undescended testis removed for repair of herniae, with "adenocarcinoma." The photomicrographs of these tumors show tubules of palisading cylindrical cells with intact basement membranes, similar to those described by Pick and others. Krückmann (23) described a case similar to Pick's in a masculine pseudohermaphrodite, with multiple tubular adenomas of the ectopic testes, absence of ovaries and marked gynecomastia. Hunt and Budd (24) reported a case of a 42-year-old man with gynecomastia, loss of libido, and a positive Aschheim-Zondek test but with a benign testicular tumor showing on analysis 20 mouse units of estrin in 1.73 grams of tumor tissue. Following orchidectomy, there was return of libido and a

reduction in the size of the breasts. The tumor was considered an interstitial cell tumor. Powell (25) mentions a benign tumor, questionably an interstitial cell tumor, in which an excess of estrin was found in the urine. Östergaard (26) recently reported a case of a 28-year-old man with gynecomastia and increased estrin in the urine without excretion of gonadotropin. No changes in sexual potentia occurred. After removal of the benign testicular tumor, the urinary estrogens fell and the gynecomastia subsided. The tumor was thought to represent an adrenal rest tumor.

Finally, Teilum (27,28) reported a case of a 58-year-old man with gynecomastia, impotence, increased urinary estrogens and a sudanophilic tubular adenoma of the testes resembling rare types of ovarian tumors mentioned by Pick and considered to be a variety of an arrhenoblastoma. Photomicrographs of this tumor bear close resemblance to the tumors described here in dogs.

In 1947 Castillo (29) and his associates presented evidence for a syndrome "produced by absence of the germinal epithelium without impairment of the Sertoli or Leydig cells." This syndrome is based on a series of 5 patients, aged 22 to 36 years, all of whom complained of sterility. These patients were normal on physical examination except for bilaterally small testes which on section were interpreted as showing small seminiferous tubules with normal basement membranes and Sertoli cells but with "complete" absence of germinal epithelium. Leydig cells appeared normal. Of primary interest to the discussion of the moment is that these individuals excreted normal amounts of follicle-stimulating hormone. Castillo and his co-workers suggest that the Sertoli cells produce a substance similar to estrogen, which regulates the production of the follicle-stimulating hormone of the pituitary. Here, as in the case of the next syndrome to be described, disease had produced a specific lesion of the germinal epithelium without apparent damage to either the Sertoli cells or cells of Leydig.

In considering testicular hormones other than masculinizing ones, mention of a second hormone commonly brings to mind a substance known as "inhibin." Based on experimental findings, Mottram and Cramer (30) in 1923 were the first to suggest that the germinal epithelium of the testis secretes a hormone distinct and independent from that of the Leydig cells. Support for this concept has come from the investigations of Martins and Rocha (31), McCullagh and Walsh (32), and Vidgoff and associates (33), as well as others. These authors have postulated that the germinal epithelium secretes a water-soluble hormone which functions in the inhibitory action of the testis on the pituitary.

In 1942 Klinefelter, Reifenstein and Albright (34) presented evidence

TABLE I*

TABULATION OF THE DIFFERENCES BETWEEN THE KLINEFELTER, REIFENSTEIN AND ALBRIGHT SYNDROME (1942), EUNUCHOIDISM WITH INCREASED F.S.H. EXCRETION, THE CASTRATION SYNDROME, AND THE SYNDROME HERE DESCRIBED

	No. 1 Klinefelter, Reifenstein, and Albright (1942)	No. 2 Eunuchoidism with increased F.S.H.	No. 3 Castrates	No. 4 Syndrome here presented
Clinical manifestations of hypoleydigism	absent or scarce	present	present	absent
Gynecomastia	present	frequently present	frequently present	absent
17-ketosteroids	normal or subnormal	reduced	reduced (usually)	reduced
F.S.H.	increased	increased	increased	normal
Azoospermia	present	present	present	present
Sertoli cells	destroyed	absent or atrophied	absent	present
Germinal epithelium	intense lesion	absent or atrophied	absent	absent
Walls of the tubules	hyalinized	absent or atrophied	absent	normal
Leydig cells	apparent hyperplasia	absent	absent	apparent hyperplasia
Testis size	small	absent or atrophied	absent	small

* Reproduced in its entirety from the article by del Castillo, Trabucco and de la Balze (29).

for the existence of a new syndrome. This syndrome, based on a study of 9 males, aged 17 to 38 years, is described as beginning in adolescence and is characterized by the findings of bilateral gynecomastia, small testes, aspermatogenesis and increased urinary gonadotropin. Histologic exami-

nation of testicular biopsy specimens in these individuals showed lesions involving the tubular elements. In the advanced stages, hyalinization of all tubular elements was noted (*both* the germinal epithelium and Sertoli cell elements) but there was no destruction of the Leydig cells. These authors postulated a "dual hormone theory of testis physiology" suggesting that the second hormone—the hormone of the germinal epithelium—is "inhibin" and that "inhibin" is analogous and probably very similar to estrin.

In a comparison of these two syndromes, that of Castillo and that of Klinefelter, the specific difference lies in the presence of Sertoli cells in the former. A hormone produced by Sertoli cells and having a pituitary-inhibiting action would explain normal values for follicle-stimulating hormone observed in the Castillo syndrome. Absence of Sertoli cells and their secretion, in the syndrome described by Klinefelter, would explain the high titer of follicle-stimulating hormone observed in his patients (Table 1).

Certainly many of the actions of estrogens are similar to those reported for "inhibin." To enumerate only a few, estrogens inhibit the increased production of gonadotropins by the pituitary of the castrate and have been observed to cause a regression of the prostate and seminal vesicles of the normal rat. Both of these actions have been reported for "inhibin."

Evidence against a second hormone of the testis, whether it is estrin or "inhibin," is based on the observation that an androgen, such as testosterone, will inhibit gonadotropin production by the pituitary. There is little doubt as to the accuracy of this observation but question has been raised as to whether testosterone will effectively inhibit pituitary gonadotropin secretion when given in physiologic amounts. In this regard the observations of Howard (35) are of interest. He observed that effective pituitary inhibition in the human could be obtained with much smaller amounts of estrogen than of androgen. Specifically, in a castrate male, 1.0 milligram of diethylstilbestrol every two days was effective in the control of hot flashes and caused a drop in urinary gonadotropins from above 96 to less than 6 mouse units per day; 50 milligrams of testosterone propionate every two days was not effective in reducing gonadotropins to 52 mouse units per day, the upper limit of the normal range.

SUMMARY

Evidence is presented to substantiate the findings of others that, in the dog, feminizing testicular tumors exist which appear to be composed of Sertoli cells, are rich in lipids, and produce considerable quantities of estrogen. This evidence combined with other studies in humans in which estrogen production appears to be related to the presence of Sertoli cells,

forms the basis for the postulate that the second hormone of the testis is an estrogen produced by the sustentacular cells of Sertoli.

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METABOLIC ACTIONS AND FATE OF INTRAVENOUSLY ADMINISTERED ADRENOCORTICOTROPIC* HORMONE IN MAN†§

GEORGE SAYERS, PH.D., THOMAS W. BURNS, M.D., FRANK H. TYLER, M.D., B. V. JAGER, M.D., THEODORE B. SCHWARTZ, M.D., EMIL L. SMITH, PH.D., L. T. SAMUELS, PH.D. AND HORACE W. DAVENPORT, PH.D.

From the Departments of Pharmacology, Medicine, Biochemistry and Physiology, and the Laboratory for the Study of Hereditary and Metabolic Disorders, University of Utah College of Medicine, Salt Lake City, Utah

THE metabolic alterations following the administration of pituitary adrenocorticotropic hormone (ACTH) by the intramuscular or subcutaneous route have been reported by a number of investigators (1, 2, 3). The present study concerns the metabolic actions and fate of single large doses of ACTH injected intravenously. Special attention has been paid to the temporal relationship between the dynamic changes which occurred in certain constituents of the body fluids and the titer of tropic hormone in the plasma. Renal excretion of the administered hormone has also been studied.

MATERIAL AND METHODS

The 2 subjects of these experiments were healthy male medical students; M.L. was 26 years old and weighed 84 Kg., and M.J. was 29 years old and weighed 89 Kg. They were on a controlled diet and lived in the metabolic ward of the Department of Medicine for a number of days before and during the hormone studies. Subject M.L. consumed 3100 calories per day which included 360 Gm. of carbohydrate. Subject M.J. received 2450 calories per day which included 260 Gm. of carbohydrate. Breakfast was withheld from both subjects on the morning of the experiments, but a meal was given six hours following the beginning of hormone administration. For M.L. this meal contained 100 Gm. of carbohydrate; for M.J., 80 Gm. of carbohydrate. Both subjects were again fed during the tenth hour. This meal contained 150 Gm. of carbohydrate for M.L. and 110 Gm. for M.J.

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In order to insure adequate urine volumes, the subjects ingested ample amounts of fluid starting two hours before and continuing until six hours after ACTH administration. Inclusive of the volume infused during this period, M.L. had a total fluid intake of 1800 ml.; M.J., 1200 ml.

Two preparations of ACTH[1] were employed. The properties of these products, presented in Table 1, show that they are potent adrenocorticotropins which contain only traces of other pituitary hormone activities. M.L. received 240 mg. of one preparation (37-KE), an amount equivalent

TABLE 1. BIOLOGIC PROPERTIES AND DOSES OF THE ACTH PREPARATIONS

	Preparation 37-KE	*Preparation 42-B(G-59703)*
ACTH activity	—41.5 ± 12 per cent of Armour standard La-1-A*	—16 ± 4 per cent of Armour standard La-1-A
Posterior pituitary hormones	—oxytocic—0.0025 U.S.P. unit per mg.	—0.0036 unit per mg.
	—pressor—0.005 unit per mg.	—0.0045 unit per mg.
Prolactin	—0.5 unit per mg.	—1 unit per mg.
Growth hormone	—negligible	—negligible
Gonadotropins	—2.0 Collip units per mg.	—1.5 Collip units per mg.
Thyrotropin	—0.037 ± 0.06 Evans chick unit per mg.	— ?
Moisture	—5.96 per cent	— ?
Administered to	—M.L.	—M.J.
Total amount infused	—240 mg.	—330 mg.
Total ACTH activity infused equivalent to La-1-A	—100 mg.	—50 mg.

* Highly potent Armour ACTH standard.

in ACTH activity to 100 mg. of a purified standard (La-1-A, Armour); M.J. received 330 mg. of the other preparation (42-B), an amount equivalent in ACTH activity to 50 mg. of the same standard. Despite the large doses administered, evidence of pressor activity was not observed in either subject; inhibition of diuresis, a most sensitive index of posterior pituitary activity,[2] occurred in M.L. only.

Before the infusion of ACTH was started, both subjects were examined for their sensitivity to the ACTH preparations by ophthalmic and intradermal tests, and gave negative responses. Moreover, when reexamined by the same tests six weeks after the experiment, neither subject showed any evidence of acquired sensitivity.

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[1] Kindly supplied by Dr. John Mote of the Armour Laboratories.

[2] Quantities as small as 0.5 pressor unit injected intramuscularly will produce a marked inhibition of water diuresis (4).

The hormone preparations were infused in 200 ml. of physiologic saline. For the first subject, M.L., the time of infusion was one hour, but approximately 75 per cent of the total amount was given at a constant rate during the last half-hour. In the case of M.J., the entire 200 ml. was infused at a constant rate over a period of one-half hour.

For ACTH determinations, plasma and urine samples were shell frozen ready for lyophilization within a period of one hour following their procurement. Studies of two of the authors (G. S. and T. W. B.) have shown that the procedure is effective for preserving ACTH activity. ACTH activity was determined by the adrenal ascorbic acid-depletion method of Sayers, Sayers and Woodbury (5).

Blood sugar was determined by the colorimetric copper method of Nelson (6) and Somogyi (7); serum phosphate, by the technic of Fiske and SubbaRow (8). Urinary nitrogen was measured by nesslerization following digestion of the sample.

The hematologic studies were conducted by routine clinical methods except for the eosinophil counts which were determined according to the method described by Forsham *et al.* (2).

Serum proteins were characterized by electrophoretic studies in a Tiselius apparatus equipped with the Longsworth schlieren scanning device. The measurements were conducted at 1° C after equilibration for forty-eight hours with diethyl barbiturate (veronal) buffer at pH 8.5 to 8.6 and at an ionic strength of 0.1. Photographs were taken of the descending boundaries only. The protein concentration in the cell was 1.5 Gm. per 100 ml.

Antistreptolysin O was measured by the method of Todd (9) as modified by Lancefield (10). Isoagglutinins, agglutinins to typhoid H and O antigens, and diphtheria antitoxin were estimated by procedures recommended by Enders (11). In subject M.L. the serum gamma globulin was measured immunologically by the quantitative precipitin technic (12). The antiserum for this determination was prepared by immunizing rabbits to a highly purified human gamma globulin.

Peptidase assays on samples of serum were performed within twenty-four hours of the withdrawal of the blood specimens. The measurements were made by the titration of liberated carboxyl groups (13). The hydrolysis of the following was studied: glycylglycine (glycylglycine dipeptidase (14)), triglycine (tripeptidase (15, 16)), glycyl-*L*-proline (prolidase (16)), and *L*-leucinamide (leucine aminopeptidase (15, 17)).

Uric acid was determined by Archibald's modification of the technic of Kern and Stransky (18) as described by Forsham *et al.* (2). The Jaffe reaction (19) was applied to the determination of creatinine and creatine.

Sodium and potassium in plasma and urine were measured in a flame

TABLE 2. PLASMA TITERS OF INTRAVENOUSLY ADMINISTERED ACTH

Subject	Time in hours following beginning of ACTH infusion	Plasma injected into test rats (ml. per 100 Gm. body weight)	No. of rats	Response as measured by reduction in ascorbic acid (mg. per 100 Gm. adrenal tissue,* av. & std. error)	Estimate of concentration of ACTH in plasma (micrograms per 100 ml.)
M.L.	0	2	4	16 ± 3	<10
	End of one hour infusion period	0.1	4	75 ± 4	250–1000
		0.5	3	124 ± 6	
	2	1.0	4	53 ± 11	25–100
	3	2	3	14 ± 6	<10
	6	2	3	24 ± 11	<10
	9	2	3	−5 ± 5†	<10
	12	2	4	14 ± 7	<10
	24	1	2	−12†	<10
M.J.	0	2	5	9 ± 6	<10
	End of 30-minute infusion period	0.5	4	111 ± 15	100–400
		2	4	131 ± 17	
	1	0.5	4	38 ± 4	50–150
		2	3	111 ± 5	
	1.5	2	4	53 ± 16	25–100
	2	2	5	31 ± 8	10–40
	3	2	7	18 ± 10	<10
	6	2	5	17 ± 7	<10

* Left adrenal removed as control. Plasma injected via tail vein. One hour later right adrenal removed. Response expressed as difference in concentration of ascorbic acid between left and right glands.

† Negative sign means concentration of ascorbic acid in right gland greater than that in left.

photometer with an internal lithium standard; chloride was determined by the method of Schales and Schales (20) with precautions regarding pH (21); plasma pH was measured on a standardized glass electrode and the CO_2 content of the plasma by the manometric technic of Van Slyke and Neill (22). Blood for the above analyses was obtained from the antecubital vein without venostasis and without exposure of the blood to air.

Urinary 17-ketosteroids were measured by the technic of Callow (23) corrected for interfering chromogens as outlined by Talbot (24). Neutral-lipid reducing substances in the urine were determined by the method of Heard, Sobel and Venning (25) which measures all steroids with a C_{20} carbonyl group, thus including desoxycorticosterone and the C_{11} oxygenated cortical hormones.

RESULTS

Clinical observations. Untoward reaction to the hormone was minimal. M.L. had a mild, shaking chill of undetermined origin, which began 75 minutes after the start of the infusion and lasted for twenty minutes. This subject's temperature was 97.5° F. at the end of the chill and one hour later had risen to 99.4° F. No significant alteration in blood pressure occurred during the course of the experiment in either subject. The post-infusion course of M.J. was entirely uneventful.

Fate of adrenocorticotropin. The ACTH content of body fluids is expressed in terms of the amount of the standard which would produce an equivalent response in the bioassay animal. Assays on control plasma and urine samples taken from both subjects before the experiment indicated that ACTH was not present in detectable amounts, *i.e.*, there was less than 10 micrograms per 100 ml.[3]

The data in Table 2 show that the concentration of hormone in the plasma of M.L. at the end of the infusion period was equal to 500 (250 to 1000) micrograms[4] per 100 ml.; two hours after the beginning of the infusion it was 50 (25 to 100) micrograms[4] per 100 ml. After the second hour, the amount of hormone in the plasma was indistinguishable from pre-infusion values. As shown in Table 2 the rate of disappearance of the hormone from the plasma of M.J. was similar to that of the first subject. The lower titers

[3] This estimate of hormone content is based upon the sensitivity of the assay method. Quantities as small as 0.2 microgram of a highly purified preparation of ACTH can be detected. Hence, when 2.0 ml. of plasma injected into the test animal give either a questionably significant or zero response, then 100 ml. of this plasma will contain an amount of hormone activity less than that of 10 micrograms of the standard (La-1-A).

[4] These titers are expressed as a range which indicates the error of the estimate of the potency. For example, the sample of plasma taken from M.L. at the end of the infusion was estimated to contain 500 micrograms per 100 ml., with a probable error of −50 to +100 per cent.

in the plasma of M.J. correspond to the smaller dose of ACTH which was given to this subject.

Samples of urine were obtained at various times during the course of the experiment. The concentration of hormone in the urine was never greater than 10 micrograms per 100 ml. of urine. It may be estimated that in each subject less than 200 micrograms of ACTH appeared in the urine during the 24-hour period following hormone administration. Unless the hormone is present in the urine in some inactive form, renal excretion can be disregarded as a significant factor in the elimination of ACTH from the body.

The very rapid disappearance of the administered hormone from circulating plasma may be due to inactivation and/or rapid diffusion of the

TABLE 3. ALTERATIONS IN BODY FLUID CONSTITUENTS ASSOCIATED WITH GLUCONEOGENESIS

Subject	Time in hours following beginning of ACTH infusion	Blood sugar, mg. per 100 ml.	Serum phosphate, mg. per 100 ml.	Urine collection periods*	Urinary phosphate excretion, mg. per mg. creatinine	Urinary nitrogen, mg. N per mg. creatinine per min.
M.L.	0	98	4.8	−1– 0	—	6.2
	1	118	—	0– 1	—	4.9
	2	96	4.1	1– 2	—	6.3
	3	105	4.1	2– 3	—	6.8
	4	103	3.9	3– 4	—	7.6
	5	112	4.5	4– 5	—	9.7
	6 (Meal 6.0)	96	4.8	5– 6	—	5.8
	9 (Meal 9.5)	205	4.1	6– 9	—	5.7
	12	103	4.6	9–12	—	4.8
	24	100	4.4	12–24	—	4.8
M.J.	0	82	3.5	control 24 hours	0.39	6.7
	2	78	3.5	0– 3	0.26	7.5
	3	93	4.0	3– 6	0.37	16.3
	6 (Meal 6.0)	97	3.9			
	6.7	162	3.6			
	7.1	197	3.3	6–12	0.39	7.7
	7.9	183	4.0			
	12 (Meal 9.5)	104	—	12–24	0.54	7.5
	24	85	—			

* The numbers in this column indicate hours following the beginning of ACTH infusion.

TABLE 4. HEMATOLOGIC CHANGES

Subject	Time in hours following beginning of ACTH infusion	Total white count, cells per cu. mm.	Neutrophils, cells per cu. mm.	Lymphocytes, cells per cu. mm.	Eosinophils, cells per cu. mm.	Platelets per cu. mm.	Hematocrit, ml. packed r.b.c. per 100 ml. blood	Erythrocyte sedimentation rate, cu. mm. per hr.
M.L.	0	4,950	1,930	2,520	57	—	45	2.5
	1	3,250	1,720	1,200	8	—	46	8
	2	5,950	4,450	475	22	—	46	—
	3	9,800	7,120	784	0	—	45	20
	4	11,050	7,820	884	19	—	46	3
	5	—	—	—	3	—	46	6
	6	9,350	5,800	2,710	3	—	46	3
	9	9,950	6,760	2,490	10	—	46	—
	12	—	—	—	61	—	46	5
	24	4,500	2,120	1,710	67	—	45	6
M.J.	0	9,150	5,120	3,610	200	220,000	52	0
	2	8,100	4,860	1,540	108	210,000	48	0
	3	9,650	6,380	1,540	97	140,000	49	2
	6	11,450	7,900	1,490	11	125,000	49	2
	7	—	—	—	8	—	—	—
	12	13,650	6,410	5,740	100	—	41	—
	24	9,000	3,780	3,440	186	270,000	38	2

hormone into the tissues. Because ACTH could conceivably be excreted in the urine in a conjugated inactive form and since the activity of certain preparations of ACTH can be enhanced by acid treatment,[5] the urine of subject M.L. was acidified to pH 1.0; however, no increase in ACTH activity was found. In contrast to ACTH, gonadotropin, after a single injection in the human, is excreted in the urine in appreciable quantities for a number of days (26). The ACTH preparations employed in the present study were isolated from hog pituitaries; one cannot be certain that the ACTH elaborated by the human pituitary is subject to the same fate as the porcine hormone.

Blood sugar, serum phosphate, and urinary phosphate and nitrogen. No significant change occurred in the fasting blood glucose (Table 3). Following a meal of 80–100 Gm. of carbohydrate, both individuals had glycosuria exceeding two per cent and abnormally high blood glucose levels. Glucose was no longer detectable in the 9–12 hour sample of urine of both subjects and blood sugar values returned to normal in spite of the ingestion of a

[5] Unpublished data of T. W. B. and G. S.

second large carbohydrate meal in the interval. Both subjects showed increased total urinary nitrogen excretion in the early period after ACTH administration, a fact consistent with the view that carbohydrate was being formed at the expense of tissue protein. These metabolic alterations are typical of the well-known gluconeogenic action of cortical steroids with an oxygen on C-11. The results confirm and extend the findings of Forsham *et al.* (2) who showed somewhat comparable changes following chronic administration of ACTH. Thus the metabolic evidence suggests that ACTH brings about an increased secretion of steroids with an oxygen on C-11 from the adrenal cortex.

Serum and urinary inorganic phosphate changes were not sufficiently definite or consistent to warrant any conclusions based on them.

Formed elements of the blood. In both subjects the total white blood cell count, which was initially normal, became elevated because of an increase in the number of circulating neutrophils (Table 4). In contrast, the number of circulating lymphocytes markedly decreased and within a period of two hours reached a minimal level which persisted through the fourth to sixth hours. By the ninth hour the lymphocyte count was either normal or above normal. In both subjects, the number of circulating eosinophils fell markedly and reached a minimum at five to seven hours after administration of hormone. By the twelfth hour the number of eosinophils was either normal or approaching normal and by the twenty-fourth hour had definitely returned to normal.

In the case of M.L., despite the withdrawal of more than 500 ml. of blood, the volume of packed red blood cells remained unchanged over a period of twenty-four hours (Table 4). The volume of packed red blood cells of M.J. from whom 600 ml. of blood was withdrawn remained relatively constant during the first six hours of the experiment and then declined from 52 to 38 ml. per 100 ml. of blood at the end of twenty-four hours.

In subject M.L., there was a suggestive increase in the erythrocyte sedimentation rate at the third hour. For this reason serial estimations of plasma fibrinogen (method of Cullen and Van Slyke (27)) were made on subject M.J. However, no change occurred in the erythrocyte sedimentation rate or the fibrinogen concentration of the plasma of subject M.J. It is probable that the increased sedimentation rate in M.L. was related to the chill and fever which he experienced rather than to an effect of ACTH mediated by the adrenal cortex.

Serum iron and copper were determined on samples of blood taken immediately before, and at six and seven hours following, the administration of ACTH. No significant alteration occurred in the serum concentrations of these elements.

The changes in the cellular elements of the blood confirm the experi-

ments of White and Dougherty in animals (28) and of Forsham *et al.* (2) in human subjects. The elevation of the number of circulating neutrophils is probably not a result of activation of the adrenal cortex, since this same effect can be obtained with ACTH in adrenalectomized animals (28) and in patients with Addison's disease (2). Because ACTH does not produce a lymphocytopenia in adrenalectomized animals (28) or in patients with adrenal insufficiency (2), it is fairly certain that the lymphocytopenia is mediated by the adrenal cortex, in particular, by the action of cortical steroids which have gluconeogenic properties. Similarly, the decrease in the number of circulating eosinophils which follows administration of ACTH has been shown by Forsham and associates (2) to depend upon the presence of functioning adrenal cortical tissue.

Serum proteins. Samples of blood were taken from M.L. at 0, 3, 6, 12, 24, 48 and 96 hours. The electrophoretic distribution of serum proteins was normal at all these times. The $\alpha_2+\alpha_1$ globulins were found to be 16 ± 1 per cent; $\beta_2+\beta_1$, 11 ± 1 per cent; $\gamma_2+\gamma_1$, 14 ± 2 per cent; and albumin, 59 ± 3 per cent. Samples of serum were taken from M.J. at 0, 3, 6, 12 and 24 hours. Again, distribution of the serum proteins was normal and remained unchanged throughout the entire series. Values obtained were as follows: $\alpha_2+\alpha_1$, 11 ± 1 per cent; $\beta_2+\beta_1$, 11 ± 1 per cent; $\gamma_2+\gamma_1$, 14 ± 2 per cent; and albumin, 64 ± 1 per cent. It should be noted that although the α-globulin concentration differed markedly in the two subjects, there was no significant change in either case following injection of ACTH.

In patient M.L., the "gamma globulin," as estimated immunologically, ranged from 27 to 31 per cent of the total serum protein. The values for gamma globulin as estimated immunologically (12) are greatly in excess of the values obtained electrophoretically. The control value was 30 per cent and a maximum value of 31 per cent was attained at the third hour; thereafter, the value ranged between 27 and 28 per cent of the total serum protein, up to and including the ninety-sixth hour. Such alterations as were noted in this protein fraction after administration of ACTH do not appear significant.

Immune bodies. M.L. had an elevated antistreptolysin O titer (200 units) during the control period. The titer did not change appreciably in the serial estimations performed during the seventy-two hours after ACTH administration. Although this subject twice received typhoid vaccine in the previous three years, he had no demonstrable antibodies to H and O antigens during the control period or subsequent to ACTH injection.

M.J. also had an elevated antistreptolysin O titer (250 units) prior to injection of ACTH and showed no increase subsequently. This subject had received typhoid vaccine in the past three years and, in addition, received 1.0 ml. of typhoid vaccine subcutaneously five days before the experiment.

TABLE 5. EFFECT OF ACTH ON SERUM PEPTIDASES

The substrates (0.05 M) were glycylglycine (gg) in the presence of 0.001 M Co^{++}, glycylglycylglycine (ggg), *L*-leucinamide (la) in the presence of 0.001 M Mn^{++}, and glycyl-*L*-proline (gp) in the presence of 0.001 M Mn^{++}. The solutions were buffered with 0.025 M veronal at pH 7.8. The total volume of each mixture was 2.5 ml., and hydrolysis was determined on 0.2 ml. samples by the titration of liberated carboxyl groups by the method of Grassmann and Heyde (13). The results are expressed as $K \times 10^4$, the first order velocity constant for 0.2 ml. serum per ml. of reaction mixture. Those values which appear to differ significantly from the control values are in bold-face.

Subject	Time in hours following beginning of ACTH infusion	gg	ggg	la	gp
M.L.	0	2.2	3.1	0.3	4.6
	3	**3.5**	**3.9**	0.5	3.4
	6	**4.9**	**4.2**	0.3	4.3
	12	1.9	2.7	0.4	4.4
	24	2.0	3.3	0.8	3.5
	48	2.0	2.7	0.7	3.8
	96	2.6	2.9	0.4	2.9
M.J.	−24	4.5	3.0	0.5	2.6
	0	4.5	3.7	0.4	3.4
	3	4.5	3.3	0.5	**4.0**
	6	4.8	**6.0**	0.5	2.8
	24	4.1	4.1	0.5	3.0

No antibodies to the H and O antigens could be demonstrated in his serum taken immediately prior to or at intervals during the twenty-four hours following ACTH injection. The isoagglutinin titer of this subject (Group O) to group A and B cells did not change throughout the course of the experiment. The control diphtheria antitoxin level of 0.6 unit in his serum, as measured by the rabbit skin test, did not change significantly during the ninety-six hours following ACTH administration.

The failure of the gamma globulins and serum antibodies to rise in the normal human following administration of ACTH is in contrast to the experimental results of White and Dougherty (29) in rabbits. However, our results confirm the negative findings of Forsham *et al.* (2) and of Herbert and deVries (30) in human subjects. The evidence available at present, from experiments in which large doses of ACTH are given, appears to indicate that in the human the secretory activity of the adrenal cortex can play no more than a minor role in the mobilization of antibodies from tissue stores. It is possible that only a meager quantity of antibodies is stored in the human, so that no significant increase in their serum level could be expected following administration of ACTH.

Serum peptidases. The activity of several serum peptidases was measured after ACTH injection in order to determine whether changes occurred in humans similar to those reported in mice by Holman, White, and Fruton (31). These investigators measured the activity of leucine aminopeptidase (substrate: *L*-leucinamide), and of a tripeptide-splitting enzyme (substrate: *L*-leucylglycylglycine) before and after the administration of cortical extracts and of ACTH. Cortical extracts significantly increased the level of both enzymes; ACTH increased only the tripeptidase level.

The conditions of the measurements and the results are given in Table 5. It is apparent that a definite increase in the hydrolytic activity of the serum of M.L. occurred at three and six hours, as measured with glycylglycine (gg) and glycylglycylglycine (ggg); the level of activity returned to normal by the twelfth hour. The rate of hydrolysis of glycylproline (gp) and of leucinamide were not significantly altered.

Small but insignificant changes in the rates of hydrolysis of gg and ggg by the sera of M.J. appear to have taken place at six hours when com-

TABLE 6. ALTERATIONS IN SERUM URIC ACID, URINE URIC ACID, CREATINE AND CREATININE

Subject	Urine collection periods*	Serum uric acid, mg./100 ml. at beginning of period	Urine creatinine, mg. excreted per minute	Urine uric acid, mg. excreted per minute	Ratio uric acid / creatinine	Per cent change in UA/C
M.L.	Control	3.9	1.18	0.43	0.36	
	0– 1	—	1.11	0.58	0.53	+47
	1– 2	3.7	1.08	0.71	0.66	+83
	2– 3	3.7	1.45	0.81	0.56	+56
	3– 4	4.0	1.18	0.48	0.41	+14
	4– 5	4.0	0.97	0.32	0.33	− 8
	5– 6 (Meal 6.0)	4.0	1.25	0.44	0.35	− 3
	6– 9 (Meal 9.5)	5.0	1.30	0.70	0.54	+50
	9–12	4.0	1.43	0.31	0.22	−39
	12–24	3.7	1.30	0.32	0.25	−31
M.J.	Control	5.9	1.35	0.43	0.32	
	0– 3	6.2	1.35	0.64	0.47	+47
	3– 6 (Meal 6.0)	6.6	1.31	0.55	0.42	+31
	6–12 (Meal 9.5)	6.3	0.85†	0.15	0.18	−44
	12–24	6.1	1.36	0.28	0.20	−37

* The numbers in this column indicate hours following the beginning of ACTH infusion.

† This collection appears to be incomplete.

pared to the rates before injection of ACTH and subsequent to six hours. With gp, a slight increase which does not appear significant was evident at three hours, but the rate at the sixth hour returned to the control value. The ability to hydrolyze ggg showed a marked increase at six hours. Again, the level of leucine aminopeptidase activity did not show any change.

The above data confirm, at least in part, the observation by Holman and associates (31) that adrenal cortical stimulation causes a rise in the level of certain serum peptidases. In confirmation of their results with lgg, we found a small but apparently significant rise in tripeptide (ggg) hydrolysis. There was no detectable change in either of our subjects in leucine aminopeptidase or prolidase activity. However, in the serum of M.L., a fairly marked change occurred in gg dipeptidase activity, but only a slight change was found in the serum of M.J. The small and variable changes observed in the serum peptidases do not appear to be very striking, especially in comparison with the marked decrease observed in the circulating lymphocytes and eosinophils.

Serum uric acid and urinary uric acid, creatinine and creatine. The results are presented in Table 6. The most marked alteration in these constituents was the increased rate of excretion of uric acid. Values for uric acid in the urine are expressed as ratios of uric acid to creatinine. The peak effect appeared in the second hour. The result confirms the observations of Forsham and co-workers (2). The temporal association of lymphocytopenia and increased uric acid excretion suggests that lysis of lymphocytes may be the factor responsible for the increased output of uric acid in the urine. However, further analysis of the data suggests that two processes are occurring simultaneously. The serum uric acid concentration did not fall during the period of increased urinary excretion; indeed, it rose somewhat. This suggests that both increased uric acid production and increased urinary excretion took place, an interpretation which gains support from the fact that a phase of uric acid retention occurred after most of the other metabolic changes had disappeared.

Plasma pH and CO_2, and plasma and urinary sodium, potassium and chloride. In subject M.L., there was a definite elevation in plasma pH at the second hour; the pH returned to normal by the sixth hour (Table 7). Unfortunately, no studies were made in this subject to determine the CO_2 content of the plasma. The plasma of subject M.J. underwent no change from normal values for venous blood in pH, CO_2 content or pCO_2 up to the sixth hour of the experiment, at which time the observations were terminated.

Cluxton and associates (32) have shown that in certain patients with Cushing's syndrome there is an elevation of plasma pH and CO_2 content.

A similar elevation in pH took place in subject M.L. However, no change in pH followed ACTH administration in subject M.J. and the pH change in M.L. was temporally associated with his chill and fever and disappeared before the other metabolic changes reached their peak. For these reasons, it seems likely that the alteration in pH was the result of hyperventilation accompanying the chill and fever and not a result of adrenal cortical stimulation by ACTH.

Both subjects were studied for an antidiuretic effect following ACTH administration. None was found in M.J. However, injection of ACTH

TABLE 7. ALTERATIONS IN pH AND CO_2 OF VENOUS PLASMA

Subject	Time in hours following beginning of ACTH infusion	pH	Bicarbonate, mM. per liter	pCO_2, mm. of mercury
M.L.	0	7.37		
	1 chill	7.46		
	2	7.60		
	3	7.53		
	6	7.33		
	9	7.38		
	12	7.33		
M.J.	0	7.31	27.3	56
	1	7.36	28.0	50
	2	7.31	27.9	57
	3	7.30	27.5	58
	6	7.36	25.9	49

(preparation 37-KE) inhibited a water diuresis in M.L. This subject drank 600 ml. of water between 7:30 a.m. and 8:10 a.m. and an additional 600 ml. between 9:00 a.m. and 12:15 p.m. on the day of ACTH administration. Two hundred ml. of 0.85 per cent sodium chloride solution containing the ACTH was infused between 9:10 a.m. and 10:00 a.m. Although diuresis had started (200 ml. of urine excreted between 7:30 a.m. and 9:00 a.m.), it was soon inhibited (360 ml., between 9:00 a.m. and 12:15 p.m.) and remained so until the third hour after injection of ACTH, when it reappeared. Between the third and sixth hours, 760 ml. of urine was excreted; 400 ml. of water was ingested during this same interval. The pressor activity of the two preparations of ACTH employed in these experiments is similar (Table 1), according to animal assay. However, since M.J. received a greater quantity of total solids he was injected with a correspondingly

larger dose of pressor substance according to animal assay. Since pressor and antidiuretic activities usually parallel one another, it is surprising that there was no inhibition of water diuresis in M.J.

Both subjects were on a constant daily intake of sodium, potassium and chloride for a number of days before and after the administration of ACTH. M.L. ingested 196 mEq. of sodium and 102 mEq. of potassium per day. On the day of the experiment he received an additional 29 mEq. of sodium in the infusion fluid containing the ACTH. During the first six hours after

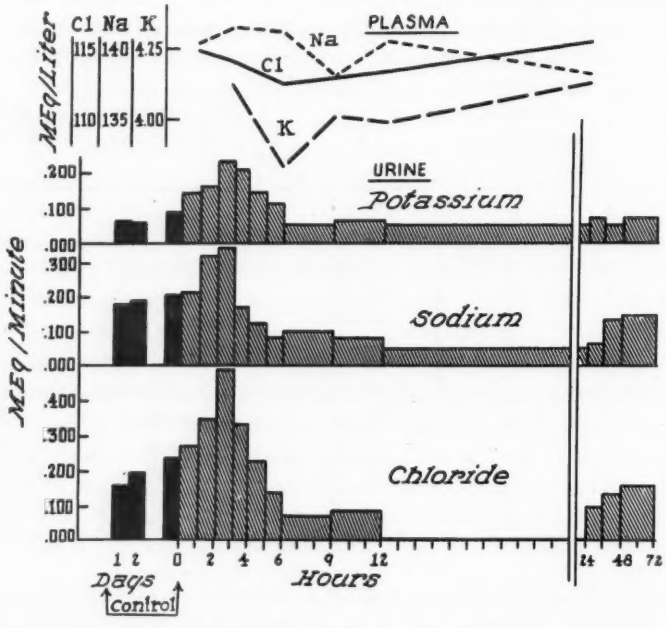

FIG. 1. Electrolyte studies on M. L.

ACTH administration 400 ml. of blood was withdrawn, which represents a loss of about 28 mEq. of sodium and 0.8 mEq. of potassium. An additional 150 ml. of blood was withdrawn between the sixth and eighteenth hours. Plasma concentrations of chloride and sodium for M.L. (Fig. 1) remained constant during the twenty-four hours following ACTH administration; plasma potassium was lowered at the sixth hour. During the first four hours after hormone administration there was an elevation in the rate of urinary excretion of chloride, sodium and potassium. From this time on, there was a marked retention of sodium and chloride which persisted at least to the thirty-sixth hour. When account is taken of all

sources of sodium gain and loss, there was an over-all retention of about 100 mEq. of sodium during the twenty-four hours following ACTH injection, as compared with the control periods. On the other hand, the rate of urinary excretion of potassium was elevated for the first six hours, returned to control levels by the seventh hour and remained there through the seventy-second hour, when observations were terminated.

M.J. ingested 127 mEq. of sodium and 97 mEq. of potassium per day and on the day of the experiment received an additional 29 mEq. of sodium

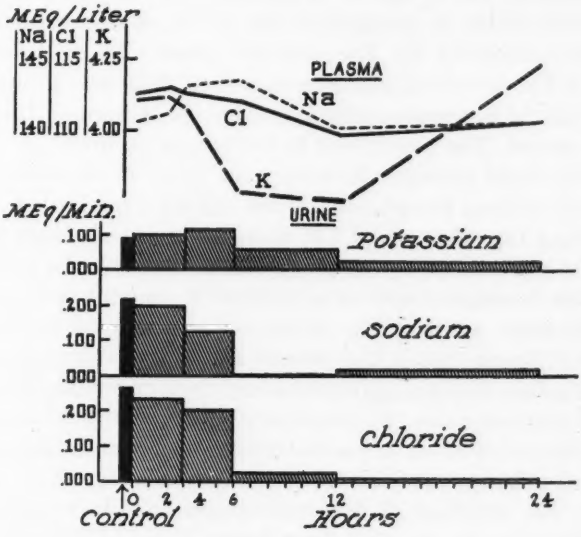

FIG. 2. Electrolyte studies on M. J.

in the infusion fluid containing the ACTH. About 29 mEq. of sodium and one mEq. of potassium were lost by removal of blood during the first six hours of the experiment. An additional 18 mEq. of sodium was withdrawn during the sixth to eighteenth hours. Plasma concentrations of sodium and chloride showed no significant change during the twenty-four hours after ACTH administration (Fig. 2). However, the plasma potassium level was depressed between the sixth and twelfth hours. The rate of urinary excretion of sodium and chloride was moderately depressed during the first six hours and markedly decreased during the next eighteen hours. There was an over-all retention of about 200 mEq. of sodium during the twenty-four hours following ACTH administration as compared with the control period. On the other hand, the rate of urinary excretion of potassium during the first six hours was slightly elevated above the pre-injection

value. From the sixth to the twelfth hour the rate of urinary potassium excretion was reduced, but not in proportion to the marked reduction in urinary excretion of sodium and chloride.

The transient sodiumphoresis and chloruresis in M.L. were associated in time with the inhibition of water diuresis in this subject. All three effects were probably due to the action of posterior pituitary antidiuretic principle, which contaminated the particular ACTH preparation. The observed reduction in plasma potassium, elevation in rate of urinary excretion of potassium, and retention of sodium and chloride are all changes which may reasonably be assigned to the action of desoxycorticosterone-like steroids elaborated by the adrenal cortex. Tissue breakdown as measured by the increased nitrogen excretion does not account for any significant part of potassium excreted in excess of normal during the experimental period. The alterations in potassium occurred at the peak of the other metabolic changes. However, the delay in the onset of sodium retention and its long persistence are not readily explained. Final conclusions regarding the effect of ACTH upon electrolyte balance must await the results of studies employing preparations in which the posterior pituitary principle is either absent or so reduced in amount that it exerts negligible physiologic actions. The preparation employed in M.J. did not inhibit water diuresis but it had pressor action by laboratory assay. The lack of parallelism between antidiuretic and pressor potency is unexpected.

Forsham and associates (2) demonstrated that daily administration of ACTH in humans resulted in marked retention of sodium and chloride and an increased urinary excretion of potassium, effects which may be attributed to the secretion of desoxycorticosterone-like steroids from the adrenal. However, in the first four hours after ACTH administration Forsham *et al.* noted an increase in the rate of urinary excretion of sodium and chloride as well as potassium. This same pattern of action was observed by us in M.L., given a preparation with antidiuretic potency, but was not seen in M.J., given one which did not inhibit diuresis. Forsham and co-workers consider the possibility that "the increased sodium, chloride, and potassium-creatinine ratios which occurred in the first four hours following ACTH administration reflect a predominant secretion of 11-17-oxysteroids as opposed to desoxycorticosterone-like factors under which circumstances an increase in potassium excretion would be accompanied by sodium and chloride retention".

Steroids. During the 24-hour period following ACTH administration, M.L. had an elevated urinary excretion of both the neutral-lipid reducing substances of Heard, Sobel and Venning (25) and the 17-ketosteroids (Table 8). In M.J., a more detailed temporal analysis was made. The most marked elevation in the excretion of both types of urinary steroids occurred during the first six hours after ACTH administration (Table 8).

These results confirm the observations of Mason *et al.* (3) and Forsham *et al.* (2) and extend the study of urinary excretion of steroids to an examination of the effect of a single injection of ACTH. The results obtained in subject M.J. indicate not only that the adrenocorticotropic hormone has a prompt stimulatory effect on the secretory activity of the adrenal cortex but also that the enhanced secretory activity of this gland soon ceases

TABLE 8. STEROIDS IN THE BODY FLUIDS

Subject	Urine collection periods*	Urinary neutral lipid reducing steroids, mg.† per hour	Urinary 17-ketosteroids, mg.‡ per hour
M.L.	Control day 1		0.43
	Control day 2		0.51
	0–24	0.28	0.72
	Control day 3	0.11	0.43
	Control day 4	0.11	0.39
M.J.	Control day 1	0.12	0.85
	Control day 2	0.17	0.81
	Control day 3	0.10	0.62
	Control day 4	0.12	0.62
	0–3	0.50	1.49
	3–6	0.44	1.76
	6–12§	0.19	0.58
	12–24	0.16	0.59
	Control day 5		0.58

* The numbers in this column indicate hours following the beginning of ACTH infusion.

† In terms of desoxycorticosterone acetate.

‡ In terms of androsterone.

§ Collection may have been incomplete. On basis of creatinine output, estimated to be 35 per cent low.

when the circulating level of ACTH again becomes normal. These findings are in agreement with the results of experimental work in animals which has demonstrated that a single dose of ACTH causes a very prompt depletion of adrenal cholesterol and ascorbic acid and that these constituents are restored to normal within a few hours (33).

The rapid drop in the rate of excretion of steroids after the maximal excretory level is reached suggests that these substances, when acutely mobilized, have but a brief sojourn in the body. The peak rate of excretion of urinary 17-ketosteroids appears to occur later than that of the neutral-lipid reducing steroids. The neutral-lipid reducing substances are more

closely related chemically to the cortical steroids than are the 17-ketosteroids. Although there is no direct evidence to support it, the belief is generally held that the adrenal cortex itself elaborates ketosteroids having anabolic and androgenic actions (34). However, it is possible that cortical steroids undergo degradation to 17-ketosteroids in the liver or other tissues. The lag in 17-ketosteroid excretion observed in our experiments could be explained by the latter possibility.

DISCUSSION

The doses of porcine ACTH used in these experiments are greater than those required to produce maximal stimulation of the adrenal cortex. Fifty mg. injected into M.J. produced as great a response as 100 mg. in M.L. as measured by the various metabolic alterations described above. Forsham *et al.* (2) considered 25 mg. given intramuscularly as providing a near-maximum response. The large quantities were employed in order to obtain ACTH levels in body fluids which could be readily detected by the assay method utilized and which might be expected to result in appreciable urinary excretion of the tropin. Despite the fact that high plasma titers of porcine ACTH were attained in human subjects, the hormone was not found in the urine in appreciable quantities. However, this does not necessarily mean that the ACTH elaborated by the human pituitary is not excreted in the urine.

The manifold changes that followed the intravenous infusion of ACTH were of short duration. The concentration of tropin in the plasma fell to control levels in less than three hours; the urinary excretion of steroids indicates that the secretory activity of the adrenal cortex had returned to normal within six hours; the metabolic changes in most instances reached their peak before the sixth hour; and practically all the constituents of the body fluids returned to control levels by the twelfth hour. It is therefore evident that a single injection of ACTH acts promptly upon the adrenal cortex and that the effects of this action persist for only a few hours.

The results of these experiments with ACTH in human subjects lend considerable support to a unitarian concept of pituitary tropic action on the adrenal cortex. ACTH produces changes in carbohydrate and protein metabolism which are to be expected of a substance which stimulates the adrenal cortex to secrete those steroids with an oxygen on C-11 and/or C-17. Furthermore, ACTH alters electrolyte metabolism in a manner which suggests that, under its influence, compounds similar to desoxycorticosterone in metabolic activity are being secreted from the adrenal cortex. Both 17-ketosteroids and steroids with a ketol side-chain are excreted in increased quantities following the administration of ACTH. These data indicate that pituitary tropins other than ACTH are not required to explain the manifold actions of the adrenal cortex, as has been

suggested by Selye (35) and Albright (34). These investigators have suggested that the elevated androgen titers which appear to be present in body fluids when the adrenal cortex is stimulated to hyperactivity are due to the adrenal cortical secretion of androgen in response to a pituitary tropin other than ACTH. However, it is just as reasonable to suppose that the androgenic steroids result from the incomplete metabolism of cortical steroids which are secreted in abnormally high concentrations in response to excessive stimulation by ACTH itself.

It is important to know whether tissues other than the adrenal cortex respond directly to ACTH. If this is true, then certain of the metabolic changes described in this paper were not necessarily mediated via the adrenals. An almost complete answer to this question has been supplied by the experiments of Forsham *et al.* (2) who have demonstrated that a patient with Addison's disease treated with ACTH for four days failed to show the carbohydrate, electrolyte, hematologic and urinary steroid alterations which occurred when individuals with functional adrenal cortical tissue were similarly treated. It remains to be proven whether the serum peptidase changes herein reported are likewise dependent upon functional adrenal cortical tissue.

In view of the fact that large doses of ACTH were employed, the question may legitimately be asked whether the metabolic and other changes described in this report have any relevance for normal pituitary-adrenal function. It is possible that comparably high titers of ACTH never exist in human body fluids even under conditions of severe stress, when maximal discharge of ACTH can be anticipated. Although we are not prepared to deny that such large doses of ACTH may stimulate the adrenal cortex in a manner which is not physiologic, we believe the possibility is remote. Nevertheless, this point deserves further study. The following considerations suggest that the ACTH doses employed, even though maximal, are not inordinately excessive. The normal adult pituitary contains an amount of ACTH equivalent in activity to that of 20 mg. of the standard preparation employed by us (unpublished observations). The results of experiments in the rat indicate that a severe acute stress causes the release of approximately 50 per cent of the ACTH stored in the rat pituitary. If the same conditions obtain in man, then it is possible for 10 mg. (in terms of the standard) of ACTH to be released into the blood stream of a human subject within a very short period of time. The doses used in our experiments were only five- to tenfold greater than this amount and were infused over a period of one hour.

SUMMARY

1. Large single doses of pituitary ACTH were administered to 2 healthy young adults by slow intravenous infusion.

2. No change in blood pressure occurred in either subject. The only untoward reaction was a mild chill and fever experienced by one of the subjects.

3. The tropin disappeared rapidly from the plasma, reaching control levels two hours after the infusion was terminated. Negligible quantities of ACTH appeared in the urine.

4. Fasting blood sugar was not influenced by ACTH. However, glycosuria and a diabetic type of blood sugar response followed the ingestion of a meal six hours after the start of the infusion.

5. Urinary nitrogen and uric acid were elevated.

6. A marked decrease in the number of circulating lymphocytes and eosinophils occurred concomitantly with an increase in the number of circulating neutrophils.

7. The electrophoretic distribution of serum proteins was unchanged.

8. The titers of antistreptolysin O, isoagglutinins, typhoid H and O and diphtheria antibodies were uninfluenced.

9. Certain serum peptidases underwent small but inconsistent increases.

10. There was an elevation in the pH of the blood of one subject which appeared to be related to the chill and fever. There was no change in either the pH or CO_2 of the plasma of the other subject.

11. Plasma sodium and chloride concentrations remained unchanged, but plasma potassium declined significantly in both subjects at a time when the other metabolic effects were maximal.

12. There was an early increase in the urinary excretion of potassium followed by return to a normal or slightly subnormal potassium excretion from the sixth to the twenty-fourth hour. One subject showed a slight retention of sodium and chloride during the first six hours while the other subject, in whom an inhibition of a water diuresis occurred, showed sodiumphoresis and chloruresis during this same period. There was a marked retention of sodium and chloride in both subjects from the sixth to the twenty-fourth hour.

13. The quantity of 17-ketosteroids and of neutral-lipid reducing substances in the urine was markedly elevated. The peak rate of excretion of these substances occurred at about three hours after ACTH administration and their level in the urine returned to normal by the twelfth hour.

14. The sequence of metabolic and other events was such that the secretory activity of the adrenal cortex appeared to reach a maximum by the third hour after ACTH administration and to return to pretreatment activity by the sixth hour. Most of the alterations in metabolic constituents reached a maximal deviation from pretreatment levels at the sixth hour; the values returned to normal by the twelfth hour.

15. The significance of the observed results for pituitary-adrenal function is discussed.

Acknowledgments

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THE RENAL CLEARANCE OF CHORIONIC GONADOTROPIC HORMONE IN PREGNANCY, IN NEOPLASM OF THE TESTIS AND IN HYDATIDIFORM MOLE*

CLIFFORD F. GASTINEAU, M.D.,† A. ALBERT, Ph.D., M.D. AND LAWRENCE M. RANDALL, M.D.

From the Endocrinology Laboratory, Section on Clinical Physiology, and the Section on Obstetrics and Gynecology, Mayo Clinic, Rochester, Minnesota

AS IS well known, chorionic gonadotropin appears in the urine of pregnant women twenty-five to forty-five days after the first day of the last menstrual period. The rate of excretion reaches a peak at fifty to sixty days after the last menstrual period and then falls to a rather constant level, which is maintained until the hormone disappears from the urine several days after delivery. These rather remarkable variations in the excretion of chorionic gonadotropin were first clearly described by Browne and Venning (1) in 1936, and similar changes in the serum concentration of this hormone were reported by Boycott and Rowlands (2) in 1938. These authors and others have speculated whether such variations are the result of change in the rate of formation, in the rate of destruction or in the rate of excretion of the hormone. The observations that we wish to report in the present paper were obtained in order to define more precisely the role of the kidneys in the excretion of chorionic gonadotropin by determining the renal clearance of this hormone in various clinical states.

METHODS

1. *Subjects.* The patients studied consisted of 4 normal pregnant women, a man with testicular chorioma and a woman with hydatidiform mole, in none of whom was there clinical or laboratory evidence of renal disease at any time. The patients are briefly described in the appendix.[1]

2. *Collection of Specimens.* The accuracy of the twenty-four hour collection of urine required for each clearance was confirmed by creatinine

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† Fellow in Medicine, Mayo Foundation.

[1] One pregnant subject, included in our abstract (3), has been excluded from the present report because signs of toxemia subsequently developed.

determinations. Creatinine was determined by the method of Bonsnes and Taussky (4), adapted to the Coleman jr. spectrophotometer. In no instance was there evidence of a significant error in the collection of urine. Serum representative of each twenty-four hour urine specimen was prepared by pooling serums obtained at the beginning and at the end of each collection period.

3. *Bioassay of chorionic gonadotropin.* The method of Albert (5) was used.

4. *Calculations.* Renal clearance was calculated by the conventional formula UV/B, where U is the concentration of the hormone in the urine, B is its concentration in the serum and V is the volume of urine excreted per minute. The serum-to-urine ratio was calculated by dividing urine concentration into serum concentration. Creatinine coefficients were calculated by dividing the milligrams of creatinine excreted in each twenty-four hour period by the weight of the subject in kilograms.

TABLE 1. CONCENTRATION OF CHORIONIC GONADOTROPIN IN URINE AND SERUM, AND RELATED VALUES

Subject number and diagnosis	Days past last menstrual period	Urine concentration, I.U. per ml.	Gonadotropin in urine, I.U. per 24 hours	Serum concentration, I.U. per ml.	Ratio of serum to urine concentrations	Clearance (UV/B), ml. per minute	Creatinine excretion, Gm. per 24 hours	Creatinine coefficient
Subject 1	95	23.8	28,500	24.2	1.02	0.79	1.12	21.1
Normal	105	17.8	30,300	58.7	3.30	0.36	1.02	19.2
pregnancy	114	10.4	12,700	36.8	3.54	0.24	1.06	20.1
	127	8.1	11,700	29.5	3.66	0.27	0.99	18.7
	165	4.9	6,400	16.9	3.45	0.26	0.96	16.0
	213	12.1	15,100	21.1	1.74	0.50	1.03	16.9
	261	7.2	8,500	17.3	2.40	0.34	1.12	17.5
Subject 2	80	118.5	205,000	344.0	2.90	0.43	1.43	21.4
Normal	89	40.3	66,100	90.6	2.25	0.51	1.52	22.7
pregnancy	101	25.5	47,900	105.3	4.13	0.32	1.73	25.9
	131	34.4	47,100	86.6	2.52	0.38	1.66	23.7
	163	10.3	15,200	20.8	2.01	0.51	1.34	18.7
Subject 3	111	7.6	17,500	21.7	2.86	0.56	1.26	20.1
Normal	118	4.1	6,500	16.1	3.93	0.34	1.44	22.5
pregnancy	139	12.7	19,000	44.8	3.53	0.30	1.27	19.8
	204	10.8	15,500	35.4	3.28	0.33	1.32	19.5
	259	10.5	12,000	32.2	3.07	0.26	1.18	17.4
Subject 4	70	105.0	172,000	401.0	3.82	0.30	0.98	21.4
Normal	83	95.0	147,300	261.0	2.75	0.39	0.98	21.3
pregnancy	118	12.3	25,800	61.4	5.00	0.29	0.90	18.9
Subject 5	—	10.0	10,000	—	—	—	2.27	29.8
Testicular	—	9.6	9,400	28.0	2.92	0.23	2.62	34.5
tumor								
Subject 6	—	11.6	4,600	—	—	—	0.94	18.8
Hydatidi-	—	18.3	5,500	17.2	0.94	0.22	0.92	18.5
form mole	—	7.0	7,100	11.8	1.69	0.42	1.04	20.8

RESULTS

1. *Normal pregnancy.* Table 1 illustrates the values obtained in the 4 normal subjects for each of twenty determinations. In agreement with the work of Browne and Venning and of Boycott and Rowlands, high levels of the hormone in urine and serum were observed in the first trimester and relatively small amounts were present in the latter two-thirds of pregnancy. This table shows further that creatinine was excreted at a rate rather constant for each subject and that in no instance was there evidence of significant error in the collection of urine. The creatinine coefficient on the other hand tended to diminish with advancing pregnancy as body weight increased.

Table 2 shows the average clearance obtained for each subject. The clearances did not vary significantly among the 4 subjects. The average ratio of serum concentration to urine concentration of the hormone for each subject also exhibited relatively little variation between subjects.

TABLE 2. RENAL CLEARANCES OF CHORIONIC GONADOTROPIN IN NORMAL PREGNANCY

Subject	Observations	Clearances, milliliters per minute			$\frac{\text{Serum}}{\text{Urine}}$ ratios		
		Mean	S.D.*	Range	Mean	S.D.*	Range
1	7	0.39 ±0.07†	0.20	0.24–0.79	2.73 ±0.39†	1.32	1.02–3.66
2	5	0.43 ±0.04	0.08	0.32–0.51	2.76 ±0.37	0.84	2.01–4.13
3	5	0.36 ±0.05	0.12	0.26–0.56	3.33 ±0.19	0.42	2.86–3.93
4	3	0.33 ±0.03	0.05	0.29–0.39	3.86 ±0.65	1.13	2.75–5.00

* Standard deviation.
† Standard error of the mean.

Table 3 gives the mean clearances and ratios for all 4 normal subjects according to trimester. The average clearance for the first trimester was 0.41, for the second, 0.38, and for the third, 0.36 ml. per minute. The minimal clearance observed was 0.24, the maximum was 0.79, and the average for all twenty determinations in normal pregnancy was 0.38 ml. per minute. The concentration of chorionic gonadotropin in the serum was found to average 3.06 times its concentration in the urine.

2. *Testicular tumor.* A single clearance was determined on a man with a mixed adenocarcinoma and chorioma, grade 4 (Broders' method). The clearance was 0.23 ml. per minute, and the serum-to-urine ratio was 2.92, values consistent with those observed in normal pregnancy. A saline extract of the tumor assayed 220 I.U. per gram of tissue (wet), while the

serum concentration was 28 I.U. per milliliter and the urine concentration was 9.6 I.U. per milliliter. Thus the concentration of the hormone in the tumor was some eight times as great as in the serum.

3. *Hydatidiform mole.* The subject was excreting moderate amounts of chorionic gonadotropin derived from the degenerating remnant of a hydatidiform mole which was imbedded in the wall of her uterus. Clearances (Table 1) were 0.22 and 0.42 ml. per minute preoperatively and on the first day after hysterectomy the clearance was 0.32 ml. per minute. The hormone quickly disappeared from the urine, and by the second day after operation gonadotropic activity could no longer be measured.

TABLE 3. RENAL CLEARANCES OF CHORIONIC GONADOTROPIN IN NORMAL PREGNANCY

	Observations	Clearances, milliliters per minute			$\frac{\text{Serum}}{\text{Urine}}$ ratios		
		Mean	S.D.*	Range	Mean	S.D.*	Range
First trimester, 0–92 days†	4	0.41 ±0.04‡	0.09	0.30–0.51	2.93 ±0.33‡	0.65	2.25–3.82
Second trimester, 93–185 days	12	0.38 ±0.05	0.16	0.24–0.79	3.24 ±0.32	1.10	1.02–5.00
Third trimester, 186–280 days	4	0.36 ±0.05	0.10	0.26–0.50	2.62 ±0.35	0.70	1.74–3.28
Entire pregnancy, 0–280 days	20	0.38 ±0.03	0.13	0.24–0.79	3.06 ±0.19	0.84	1.02–5.00

* Standard deviation.
† Days after the first day of the last menstrual period.
‡ Standard error of the mean.

COMMENT

The two devices of renal clearance and ratios of serum-to-urine concentration were selected as the best means for portraying the activity of the kidneys in excreting chorionic gonadotropin. The concept of renal clearance is a familiar one and has been used in the study of the excretion of a large number of substances. Although no one to our knowledge has previously used renal clearances as a means of studying the excretion of hormones, data are available from which such calculations can be made. From

the work of Smith and Smith (6, 7) an average clearance of 0.85 ml. per minute is obtained from a total of thirty-nine of their determinations on normal pregnant subjects with a rather wide range of variation. The data of Taylor and Scadron (8) permit similar computations. The average clearance for twenty-three determinations on 17 normal subjects was 0.44 ml. per minute. In the present investigation, a mean clearance of 0.38 ml. per minute was obtained for normal pregnant women, a value agreeing very well with those derived from the data of Taylor and Scadron.

The ratio of the concentration of certain substances in the serum to their concentration in the urine has been shown to be a highly significant quantity by the studies of von Rhorer (9), Addis (10) and Newburgh (11). Although it is very doubtful that such a ratio for chorionic gonadotropin has the significance that it would have for urea or sodium, the concept is a useful one and therefore is used in the presentation of this material. Although the data of Smith and Smith and of Taylor and Scadron are not adequate to permit the calculation of relative concentrations of chorionic gonadotropin in the urine and in the serum, the assumption of a urinary excretion rate of 1 to 2 ml. per minute would mean that the serum concentration was found to be from 0.86 to 4.54 times that of the urine. In the present study the serum concentration was found to be from 1.02 to 5.00 times as great as the urine concentration, with an average ratio of 3.06.

It is interesting that the clearances and ratios obtained in the instances of the man who had the testicular tumor and of the woman who had the hydatidiform mole were essentially the same as for the normal pregnant woman. This would suggest that the kidneys in nonpregnant women or in men excrete chorionic gonadotropin in the same manner as do the kidneys in pregnancy.

Since the clearance of chorionic gonadotropin was much the same in all cases, it appears that variations in serum and urine concentrations must depend on changes in the rate of formation or in the rate of destruction of the hormone. Studies are in progress to evaluate the relative importance of these factors.

SUMMARY

The renal clearance of chorionic gonadotropin has been determined during pregnancy, in hydatidiform mole, and in testicular chorioma. The mean clearance in pregnancy was 0.38 ml. per minute, and closely similar values were observed in hydatidiform mole and in testicular chorioma. The constancy of values obtained in the three trimesters of pregnancy indicates that the remarkable fluctuation of chorionic gonadotropin in pregnancy is due to changes in rates of destruction or of formation and not to differences in renal excretion.

APPENDIX

Synopsis of Cases

Case 1. The patient was a white primipara, primigravida, aged 21 years, who had no history of renal disease. Blood pressure and results of urinalysis remained within normal limits throughout the course of her pregnancy, which was terminated by spontaneous delivery of a normal healthy infant.

Case 2. The patient was a white para II, gravida III, aged 20 years, who had pyuria after a spontaneous miscarriage two years before the present study. She had had a normal pregnancy terminated one year previously by spontaneous delivery of a healthy infant. During the pregnancy studied there was no evidence of renal disease or of hypertension.

Case 3. The patient was a white para II, gravida II, aged 27 years, who had had an uneventful pregnancy two years before the present study. Blood pressure and results of urinalysis remained within normal limits throughout the course of the present pregnancy, which has resulted in a healthy infant.

Case 4. The patient was a white primipara, primigravida, aged 24 years, who had had no renal disease. No abnormality of the present pregnancy has been observed to the time of this writing (August, 1948).

Case 5. The patient was a white man, aged 27 years, who had noted a nontender firm enlargement of his right testicle in December, 1947. He was seen six weeks later when the mass was estimated to be 6.5 by 5 by 5 cm. A small disk of breast tissue was palpable beneath each nipple. Orchectomy was done February 10, 1948, and a spherical nodule 3 cm. in diameter was found in this testicle. The histologic diagnosis was "mixed grade 4 adenocarcinoma and chorionepithelioma." Laboratory data of significance are as follows: 17-ketosteroids, 13.1 mg. per 24 hours; cortin-like substances, 0.76 mg. per 24 hours; estrogens, 48 rat units per 24 hours before orchectomy and 21 rat units per 24 hours after removal of the tumor.

Case 6. The patient was a para II, gravida IV, aged 36 years, who had expelled a hydatidiform mole three months before coming to the Mayo Clinic. Dilatation and curettage had been done twice, but the Friedman reaction remained positive. Total abdominal hysterectomy was done, and a small nodule of degenerating placental tissue was found embedded in the wall of the uterine fundus.

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COARCTATION OF THE AORTA ASSOCIATED WITH ABNORMAL DIGITS, OVARIAN INSUFFICIENCY AND SHORTNESS OF STATURE*

MELVIN L. GOLDMAN, M.D.,† HENRY A. SCHROEDER, M.D. AND PALMER H. FUTCHER, M.D.

From the Department of Internal Medicine and the Oscar Johnson Institute, Washington University School of Medicine, and Barnes Hospital, St. Louis, Missouri

IN describing a syndrome characterized by primary ovarian insufficiency and decreased stature, Albright, Smith and Fraser (1) noted the presence of coarctation of the aorta in 1 patient of 11. In 5 others the diastolic blood pressure was 90 mm. of mercury or more. Pich (2), Ornstein (3) and Wilkins and Fleischmann (4) each recorded a case exhibiting ovarian insufficiency, decreased stature and coarctation. Lisser and his associates (5), in a group of 25 patients with congenitally aplastic ovaries and other associated abnormalities, described coarctation of the aorta in 1 and an anomalous toe in another.

We have recently studied 9 males and 5 females affected with coarctation of the aorta (6). We were impressed with finding that 4 of the females manifested shortness of stature and abnormal digits, and that the 3 who were adults showed clinical evidence of ovarian hypofunction. Two of these exhibited diastolic blood pressures over 90 mm. of mercury.

These observations bear on any general consideration of the nature of the syndrome of so-called ovarian agenesis. They also lead one to the supposition that those affected with this syndrome should be studied carefully for the presence of coarctation of the aorta and its often serious cardiovascular complications. Hence, we are reporting the 4 patients briefly.

CASE REPORTS

Case 1, J. F., was a 24-year-old single white woman of limited intelligence who gave a history of abnormally short stature since childhood. At the age of 13 she had not menstruated and because of this, she consulted a physician who induced one menstrual period after a series of injections of unknown nature. Breast tissue had not been palpable until stilbestrol therapy had been employed for several months. Vaginal bleeding occurred at irregular intervals while the patient received stilbestrol but had ceased upon withdrawal of the drug. The patient was 56 inches in height and weighed 110 pounds. The blood pressure in the right arm was 170 mm.Hg systolic and 112 diastolic. It was not

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† National Institute of Health Postdoctorate Research Fellow.

obtainable in the legs. Axillary and pubic hair was sparse. The genitalia were infantile and the ovaries could not be palpated. No vaginal smear was made. The fourth finger of the right hand and the fourth toes of both feet were abnormally short. The tibiae showed slight bowing. The basal metabolic rate was plus 8 per cent. X-ray examination revealed the fourth right metacarpal and fourth metatarsal and phalangeal bones of both feet to be abnormally short (Fig. 1). The diagnosis of coarctation of the aorta was made on the basis of differences in blood pressure between the upper and lower extremities, the presence of collateral circulation, and notching of the ribs shown by x-ray examination.

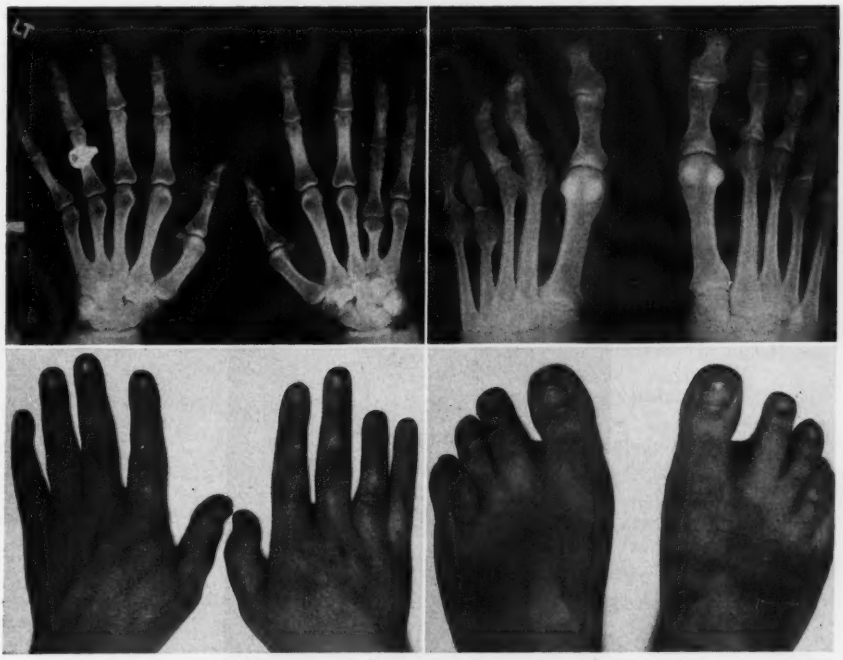

FIG. 1. Roentgenograms and photographs of the hands and feet of the patient in Case 1. There is a decrease in the length of the fourth right metacarpal bone. The shaft is narrow, the base decreased in width and the head deformed. The fourth right metatarsal is decreased somewhat in length but the phalanges are of normal size. The fourth left metatarsal and the proximal phalanx are considerably shortened.

Operative repair of the coaractation was performed by Dr. Thomas H. Burford. Menstrual function, twenty-four months later, had not returned. The blood pressure by direct puncture, eleven months postoperatively was 113 mm.Hg systolic and 75 diastolic in the right brachial artery and 132 systolic and 78 diastolic in the right femoral artery. Twenty-one months after operation, the blood pressure in the right brachial artery was 130 systolic and 75 diastolic while that in the right femoral artery was 132 systolic and 78 diastolic. The blood pressures by the ausculatatory method were 140 systolic and 80 diastolic in the arm and 150 systolic and 84 diastolic in the legs.

Case 2, O. P., was a 36-year-old colored woman who said she had menstruated once at the age of 13 years. However, her intelligence was limited and the reliability of her history is poor. Breast tissue was not present and the genitalia were infantile. Axillary and pubic hair was sparse. After therapy with stilbestrol, however, breast tissue became evident and pubic and axillary hair more abundant. Vaginal bleeding occurred at irregular intervals while the patient received this drug. She was 56 inches in height with an arm span of 65 inches and weighed 108 pounds. Her neck was short (Fig. 2). Upon examina-

FIG. 2. Photograph of the patient in Case 2 showing the short stature, short neck, and the relatively long upper extremities. The pubic hair and breast development were the result of therapy with stilbestrol.

tion of the extremities, the fourth toe of the left foot was found to be abnormally short (Fig. 3). In x-ray photographs this abnormality was found to be due to a shortening of the metatarsal and phalangeal bones. Intravenous pyelograms showed normal kidneys. Because abnormal digits, shortness of stature, and hypo-ovarianism had been seen in the other cases of coarctation of the aorta here reported, special examinations for its presence were made. The diagnosis of subclinical coarctation was established by direct measurements of arterial pressure in the upper and lower extremities and by retrograde aortic

arteriography (7). The femoral systolic pressure was 162 mm.Hg and that in the brachial artery, 193; diastolic pressure was 107 mm.Hg in the femoral artery and 116 in the brachial. Usually the femoral systolic pressure is about 20 mm.Hg higher than the brachial. The coarctation was of only mild degree (Fig. 4). In addition, the patient suffered from generalized arterial hypertension. She had a slipped epiphysis of the right femur with cystic changes in the femoral head and right acetabulum. The basal metabolic rate was plus 7 per cent. This case was similar in many respects to one reported by Stewart and Bailey, *cf.* their case number 11 (8).

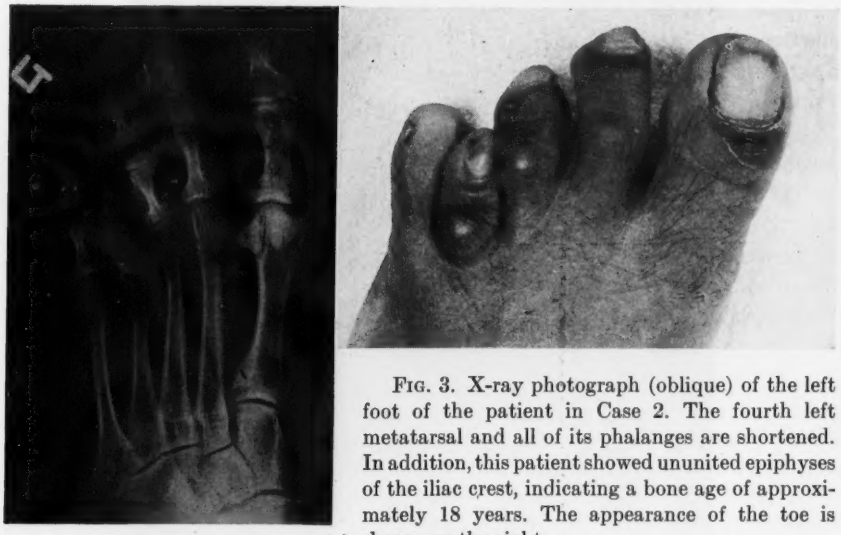

FIG. 3. X-ray photograph (oblique) of the left foot of the patient in Case 2. The fourth left metatarsal and all of its phalanges are shortened. In addition, this patient showed ununited epiphyses of the iliac crest, indicating a bone age of approximately 18 years. The appearance of the toe is shown on the right.

Case 3, N. P., was an 8-year-old white girl who complained of dyspnea of two months' duration. She had suffered three attacks of rheumatic fever with damage to her aortic and mitral valves. She was 39.5 inches in height and weighed 64 pounds. The minimum average height for an 8-year-old girl is 43 inches (9). The blood pressure in the right brachial artery by direct arterial puncture was 214 mm.Hg systolic and 57 diastolic; in the left brachial artery 124 systolic and 57 diastolic; and in the right femoral artery 87 systolic and 54 diastolic. Examination of the feet revealed short third toes which, on x-ray examination, proved to be caused by shortening of the proximal phalanges. The diagnosis of coarctation of the aorta was established by differences in blood pressure levels between the upper and lower extremities, the presence of collateral circulation, notching of the ribs in x-ray photographs, by retrograde aortic arteriography, and by exploratory thoracotomy. The subclavian artery originated at the site of the coarctation. She died at operation.

Case 4, F. R., was a 22-year-old white single woman whose chief complaints were dyspnea, fatigability and hypertension of twenty-one months' duration. A lateral nystagmus of the eyes had been present since birth. She was of limited intelligence, having been graduated from high school at age 21. She had never menstruated. She was 55 inches

in height with an arm span of 54 inches and she weighed 95 pounds. Axillary and pubic hair was scanty, and the genitalia were infantile. Breast tissue was not palpable. Stilbestrol therapy caused the breasts to develop, axillary and pubic hair to become more dense, and vaginal bleeding to occur at intervals. The blood pressure in the right brachial artery determined by direct puncture was 143 mm.Hg systolic and 60 diastolic; that in the right femoral artery was 90 systolic and 57 diastolic. The basal metabolic rate was minus 11 per cent. Examination of the extremities revealed that the fifth left finger was short-

FIG. 4. Retrograde arteriogram in Case 2, taken 3 seconds after the injection of 70 per cent diodrast into the left common carotid artery. A slight degree of constriction of the descending aorta is indicated by the arrow at the usual locality of coarctation. No collateral circulation was seen.

ened. X-ray photographs showed that the distal phalanx of this finger was rudimentary. In addition, there was failure of closure of the epiphyseal joints of the radius and ulna. Intravenous pyelograms revealed caliectasis of the left kidney. X-ray examination of the chest revealed an azygos lobe of the right lung. Diagnosis of coarctation was made on the basis of diminished blood pressure in the lower extremities as compared to the

upper, the presence of collateral circulation, notching of the ribs in x-ray photographs, and retrograde aortic arteriography. A surgical repair of the coarctation was performed by Dr. Burford in January, 1949, with excellent immediate results.

COMMENTS

The 4 females discussed in this report exhibited several of the characteristics of a general syndrome described by Turner (10), Albright and his associates (1) and others (2, 3, 4). Although determinations of urinary follicle-stimulating hormone and 17-ketosteroids were not done, presumptive evidence for hypo-ovarianism was present on the basis of developmental and menstrual history, physical findings, the scantiness of the pubic and axillary hair, and the growth of this hair following therapy with estrogens. It is probable, therefore, that these were not cases of pituitary dwarfism.

Although 4 of the 5 females in our group of 14 patients with coarctation of the aorta (6) manifested associated skeletal and sexual abnormalities, these phenomena, with the exception of shortness of stature in 3, were not observed in any of the 9 males. Webbing of the neck was not present in any case. The shortened digits were bilateral in 2 of the cases, no more than a single abnormal digit being present in any one hand or foot. In 2 of the patients, the metacarpal or metatarsal bones were primarily at fault and, in two others, the phalanges.[1] Generalized osteoporosis was not observed.

Ovarian agenesis is associated with a multiplicity of congenital defects. The cases reported here exhibited some of the anomalies noticed by others (4). Mental retardation, while not tested for, was obviously present. An eye abnormality, short neck, cystic bony changes, retardation of growth of bone, azygos lobe of the lung, and anomaly of the renal pelvis were each found. The shortening of the digits did not appear to be similar to those observed by Albright and associates (12) in pseudohypoparathyroidism (Seabright-Bantam syndrome) since in our cases only one digit of an extremity was involved. The x-ray appearance, however, resembled that seen in a case of J. S. L. Browne shown by Selye (13).

It is of interest that in Case 2 coarctation of the aorta was suspected on the basis of previous experience because of the presence of ovarian hypofunction, short stature, and abnormal digits in a patient with hypertension. Direct measurements by a Hamilton manometer of the blood pressure in the brachial and femoral arteries suggested confirmation of the diagnosis. Retrograde arteriography revealed a slight but definite degree of coarctation (Fig. 4).

[1] Albright has noted abnormal digits in 2 cases of ovarian insufficiency (11).

It is possible that the occurrence of coarctation in primary hypo-ovarianism is more frequent than previously reported, particularly in view of the common finding of slight to moderate elevation of the blood pressure in this condition (4). An alternative cause of the hypertension occurring in some patients with primary hypo-ovarianism may lie in congenital anomalies of the kidneys observed by Plachte (14) in patients with syndromes thought to be related to that of ovarian agenesis. Possibly by more specialized methods of study, such as direct measurements of arterial pressure and retrograde aortic arteriography, the milder form of coarctation might be demonstrated when these other congenital abnormalities exist.

SUMMARY

1. The occurrence of abnormal digits and shortness of stature in 4 females with coarctation of the aorta has been described. Three of the patients exhibited signs of ovarian insufficiency; the fourth patient had not reached puberty. The degree of coarctation was minimal in 1 case, requiring specialized techniques for making the diagnosis.

2. A review of the literature suggests that a slight to moderate degree of hypertension not infrequently accompanies the syndrome of primary ovarian insufficiency and decreased stature. Coarctation was reported to be demonstrable in only a few.

3. It is suggested that coarctation of the aorta should be suspected in women with primary ovarian hypofunction, abnormal digits, and an elevated blood pressure.

Acknowledgments

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OVARIAN GRANULOSA CELL TUMOR AND ACROMEGALY

HAROLD SPEERT, M.D.

From the Department of Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University, New York, N. Y.

AMENORRHEA is one of the most consistent and usually one of the earliest symptoms of pituitary tumor in women. Menstrual function may return, however, even after a prolonged period of amenorrhea, following surgical removal of the tumor, its shrinkage by roentgen irradiation, or possibly in rare instances following the escape of the expanding tumor from the sella turcica with consequent decompression of the normal elements of the gland. In the following unique case resumption of uterine bleeding occurred in an acromegalic as the result of a coincidental lesion, namely, a granulosa cell tumor of the ovary.

Case report.—The patient was a 42-year-old white, married, Russian-born housewife, who was first seen at the Columbia-Presbyterian Medical Center September 16, 1948, with the complaint of enlargement of the hands and feet and distortion of the face. These symptoms, first observed by the patient about six years before, had been preceded for about three years by excessive fatigue and frequent blurring of vision, beginning soon after the birth of her only child in 1939. More recently headache, weakness of the arms, tremors, a feeling of fullness in the mouth, and spreading of the teeth were noticed.

Her menstrual periods, which began at the age of 11½ years, had always been normal until 1945, when the patient had several profuse flows with some intermenstrual bleeding. A curettage performed at another hospital disclosed mild endometrial hyperplasia. The menses resumed their normal regularity until June 1946, when they ceased. From October 1944 to June 1948 the patient was treated intermittently with injections of estrogenic hormone because of hot flashes, fatigue, and depression, but these symptoms were much diminished for the five or six months before admission.

In January 1948 vaginal spotting occurred and the patient's physician removed a cervical polyp. In June 1948 scanty bleeding occurred for three days. Similar flows recurred in July, twice in August, and three times in September. Profuse bleeding began October 16 and continued for nine days, at the end of which time the patient was hospitalized.

Her appearance was typical of acromegaly (Fig. 1), with increase in size of the hands, feet, and facial features. Speech was thick. The optic discs showed bilateral temporal pallor but perimetric examination was unreliable because of poor cooperation. On pelvic examination a firm, freely movable, globular mass about 4 cm. in diameter was felt in the left adnexal region. The patient's weight was 61.1 Kg. and her basal metabolic rate, plus 14 per cent. Urinary estrogen excretion, determined by the fluorometric method of Jailer (1), was 32 micrograms in 24 hours. This value is higher than is normally encountered in postmenopausal women. All other chemical and serologic tests of blood, urine, and cerebrospinal fluid gave normal values. Stereoscopic roentgenologic examina-

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FIG. 1. The patient.

FIG. 2. Roentgenogram of skull, showing enlargement of sella turcica.

tion of the skull revealed a markedly enlarged sella turcica measuring 22×16 mm., with depression of its floor and thinning of the posterior clinoids (Fig. 2).

In addition to her obvious acromegaly the patient was believed to have a solid tumor of the left ovary, possibly of the granulosa cell type. On November 19, 1948, complete abdominal hysterectomy and bilateral salpingo-oophorectomy were performed. The uterus measured 9.5×4.5×3 cm. and was normal save for a slender endometrial polyp 2.8 cm. long (Fig. 3), and adenomatous hyperplasia in a thin endometrium which showed evidence of previous atrophy (Fig. 4). There was moderate squamous metaplasia in the endocervix. The right ovary was grossly normal. It contained a simple cyst about 1 cm. in diameter, several graafian follicles, and a rather dense stroma with no evidence of recent ovulation. The left ovary was replaced by a solid, encapsulated tumor measuring 3.5 cm. in diameter. It had a white, whorled appearance when cut. Microscopic sections

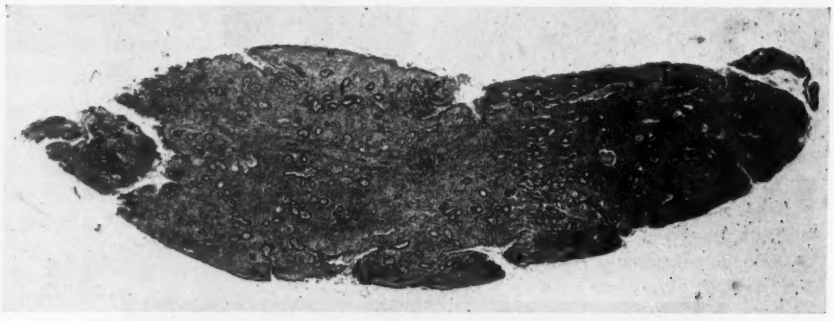

FIG. 3. Endometrial polyp.

showed a well preserved typical granulosa cell tumor. The cells were arranged in a cylindromatous pattern, with the cords of tumor cells separated by a rather dense stroma and presenting the characteristic appearance of moiré silk (Fig. 5).

Postoperative convalescence was uneventful and the patient was discharged on December 2, 1948, to continue radiotherapy to the pituitary region which was begun prior to operation.

COMMENT

Despite the high incidence of amenorrhea in women with acromegaly, there is a dearth of information concerning the genital organs of these patients (2, 3). Cushing and Davidoff (4), in their detailed treatise on the pathologic findings in acromegaly, were able to find in the literature only 4 cases in which the female pelvic organs were adequately described. More recently Henderson (5) and Klöppner (6) have written excellent papers on the sexual dysfunctions associated with adenomas of the pituitary, the former's study including 73 cases of acromegaly, without adding significantly however to the anatomic data on the genital organs. Reviews of the world's literature on acromegaly (7, 8, 9) and a recent report by Goldberg and Lisser (10) furnish a total of only about a dozen cases in

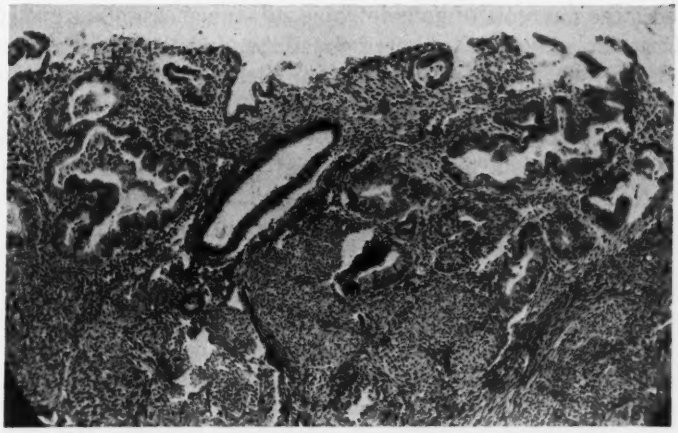

FIG. 4. Endometrium, showing adenomatous hyperplasia.

which the female reproductive organs are described. These descriptions indicate no specific ovarian pattern in acromegaly. In the elderly women, atrophy was the rule, while in the younger patients cystic changes were common. There was no clinical counterpart of the enormous enlargement of the ovaries which Putman, Benedict and Teel (11) produced in a dog by injection of pituitary extract. It is likely, however, that this extract

FIG. 5. Section of left ovary, showing granulosa cell tumor.

contained large amounts of gonadotropic substance as well as growth hormone. In acromegaly, production of gonadotropin is impaired as the result of the compression of the pituitary basophiles by the eosinophilic tumor.

The present case appears to be the first recorded instance of a granulosa cell tumor of the ovary in a patient with acromegaly. The functional activity of the tumor is attested by the high titer of urinary estrogen four months after cessation of hormonal therapy. This is adequate to explain both the irregular uterine bleeding and the adenomatous hyperplasia of the endometrium. The relation of this endometrial lesion to persistent estrogenic stimulation has been demonstrated in recent papers by Gusberg (12) and Novak and Rutledge (13). Alterations in other endocrine organs, particularly the thyroid, are fairly common in acromegaly; and Gerstel (14) has reported a case of this disorder in which several other endocrine glands were the sites of benign tumors.

The endometrial polyp in the present case may possibly be more than a coincidental finding. Despite the paucity of detailed descriptions of the female pelvic organs, this lesion has been reported in at least 3 other patients with acromegaly, aged 49, 58, and 64 years respectively (6, 10, 15). This is a higher incidence of endometrial polyps than is normally encountered in women of this age group (16) and may signify an etiologic relation to the excessive secretions of the pituitary eosinophiles.

Reece and Leonard (17) have found that estrogen inhibits the effect of growth hormone in the hypophysectomized rat. Although there is nothing in the present patient's history to indicate that remission of her acromegaly was caused by the ovarian tumor, it is interesting that a significant amelioration of her hot flashes, fatigue, and depression were observed for five or six months prior to admission.

SUMMARY

The first recorded instance of a granulosa cell tumor of the ovary in a patient with acromegaly is presented and discussed.

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ESTIMATION OF URINARY GONADOTROPIN OF THE NONPREGNANT HUMAN BY THE MOUSE UTERINE WEIGHT AND OVARIAN HYPEREMIA RESPONSES*

CHARLES W. LLOYD, M.D., MURIEL MORLEY, B.A., KATHRYN MORROW, B.A., JULIA LOBOTSKY, M.S. AND EDWARD C. HUGHES, M.D.

From the Departments of Obstetrics and Physiological Chemistry, Syracuse University College of Medicine, Syracuse, N. Y.

ESTIMATION of urinary gonadotropin excretion for clinical studies usually depends on measurement of the reaction in the gonads of immature rodents caused by administration of extracts of urine, the most widely used methods having as end-points increases in ovarian or uterine weight of rats or mice (1–6). The estimation of gonadotropin by means of measuring its power to increase the weight of the rodent uterus is a relatively sensitive procedure. However, it permits measurement only of the total gonadotropin activity because the estrogen-induced uterine enlargement can be the result either of injected luteinizing hormone or of augmentation of the animal's endogenous luteinizing hormone by administered follicle-stimulating hormone (7, 8). No simple, sensitive and accurate means of differentiating between the gonadotropins present in an extract of urine has been available for clinical use.

Since the ovarian hyperemia response in the rat is an excellent test for urinary chorionic gonadotropin (9), and since this reaction in the rat results from the action of chorionic gonadotropin as a luteinizing hormone (10), it seemed possible that the hyperemia response could be used as the basis of a test for luteinizing hormone in the urine of nonpregnant humans. Farris (11) has reported the production of ovarian hyperemia in the rat by injection of urine obtained from women on the days just preceding ovulation. Riley, Smith and Brown (12) and Kupperman and associates (13) have shown that this reaction is caused by pituitary luteinizing hormone or luteotropin and not by follicle-stimulating hormone. It, therefore, seems probable that the hyperemia reaction following injection of urinary gonadotropin results from the luteinizing hormone and/or the luteotropin content and not from the follicle-stimulating activity. This report presents data on the excretion by humans of gonadotropin as measured a) by the

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uterine weight method, which is regarded as an estimate of the total gonadotropin, and b) the ovarian hyperemia method, which is considered an estimate of luteinizing hormone or of luteotropin.[1]

METHODS

A. *Collection of urine*

Forty-eight, or occasionally 24-hour urine specimens, collected without preservative but with the urine stored at ice-box temperature during the collection period, are used.

B. *Preparation of extract:*[2]

1. Measure urine volume. Acidify to litmus, filter and add 1 Gm. NaCl per 100 cc. of urine.
2. Add 4 volumes 95% ethyl alcohol; shake well; let stand in refrigerator overnight. Remove supernatant by siphoning and discard. Centrifuge; decant and discard supernatant. Wash precipitate with ether (about 50 cc.). Centrifuge; decant and discard supernatant.
3. Dissolve precipitate in distilled water. Dialyze whole precipitate (against running tap water) in cellophane bag overnight. Pour dialyzed precipitate into centrifuge cup and thoroughly rinse out cellophane bag with distilled water.
4. Centrifuge; decant, saving supernatant. Rinse precipitate and centrifuge again. Pool supernatants.
5. Lyophilize in two equal parts (24-hour aliquots).

C. *Total gonadotropin assay:*

Two 21-day-old female white mice are used for each dosage level. Total gonadotropin excretion is expressed in terms of mouse uterine units (m.u.u.) per 24 hours. One m.u.u. is that amount of material which will cause an increase in uterine weight of 100 per cent over the uterine weights of saline injected controls. Serial dilutions of the extract are made until the smallest amount of material causing a positive result is found. Ovarian weights are also measured. An ovarian weight increase of over 100 per cent indicates relatively large amounts of F.S.H.

1. Dissolve the 24-hour extract in a convenient volume of isotonic saline solution (6 cc.).
2. Make up solutions of extract to be given to 2 mice at each dose level. The usual beginning assay levels are 8, 16, 32, and 64 m.u.u. Therefore pipette into 15 cc.

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[1] We wish to thank Dr. W. W. Westerfeld for his advice in setting up this method.

[2] The extracts used in this study were prepared by the alcohol precipitation method described. Preliminary studies have shown that the hyperemia-producing material, as well as the gonadotropins which cause a mouse uterine weight increase, can be as effectively extracted from urine by ultrafiltration (8, 14) as by alcohol precipitation. By the use of the ultrafilter, an ovarian hyperemia assay can be completed in less than twelve hours after receipt of the urine specimen.

centrifuge tubes, aliquots of 1/4, 1/8, 1/16, and 1/32 of the total extract and make up the volume with saline solution to 3 cc. in each tube.

3. Inject subcutaneously 0.25 cc. of solution per mouse twice daily for three days. Injections are at least eight hours apart.
4. Kill animals on fourth day by a blow on the head.
5. Weigh animals to nearest 0.5 Gm. Discard animals which are very heavy or very light. Weight range is from 8 to 15 Gm.
6. Remove ovaries and uterus including cervix. Dissect uterus and ovaries cleanly. Split uterus longitudinally. Blot gently and weigh to nearest 0.1 mg. on torsion balance. Blot gently and weigh ovaries together. A uterine weight to body weight ratio of 2 mg./Gm. or greater is considered positive. Average control ovarian weight is 3 mg. and a weight of over 6 mg. is called positive. At least 4 control animals are killed on each assay day.
7. If the test is positive at all levels run, further serial dilutions are made until a negative level is reached. Normal values: 4–64 m.u.u./24 hours for adult males and females.

D. *Hyperemia assay:*

The 21-day-old mouse has been used in these studies instead of the rat because the hyperemia reaction is at least 10 to 20 times more sensitive in the mouse than in the rat when urinary gonadotropin is administered. Our observations confirm those of Zondek *et al.* (10), who found spontaneous hyperemia reactions more frequent in the mouse. By proper precautions to be described later it has been possible to keep the incidence of these reactions at a very low level which does not interfere with the usefuleness of the technique.

1. Dissolve the 24-hour urine extract in a convenient volume of isotonic saline solution (6 cc.).
2. Make up solutions of extract to be given to 4 mice at each dose level. Dilutions routinely used for the initial assay are 1/16, 1/64, 1/128, and 1/256 of the 24-hour extract for each mouse. Each dose is given in one injection in a volume of 1 cc.
3. Give subcutaneously each dilution of extract to one mouse. This is the preliminary "range finding" assay. The results of this "range finding" indicate the dilutions to be administered to 3 additional animals in order to complete the assay on the following day.
4. Kill the mice with carbon monoxide seven hours after injection.
5. Remove the ovaries, including enough of the mesentery and horn of the uterus to prevent drainage of blood from the ovarian veins. The reaction is read by evaluating the color response of the ovary. Three types of reaction are encountered: a) Positive: the ovary is as red as kidney. b) Negative: the ovary is pale white or very slightly pink. c) Equivocal: the ovary is neither as red as kidney nor sufficiently pale to be called "negative." The incidence of these reactions in saline injected mice is as follows:

No. of mice	No. of ovaries	Plus	Plus minus	Minus
127	254	22(8.6%)	95(37.4%)	137(54%)

A dosage level is called positive when 2 (25%) of the 8 ovaries of the 4 mice receiving that dose show a positive result. A mouse hyperemia unit (m.h.u.) is defined as the amount of extract causing 25% of 8 ovaries to have a "positive" reaction. The "equivocal" reaction is considered "negative" in interpreting the test.

At least 4 saline injected control animals are sacrificed on each day that assays are performed.

Care of animals:

Immature female white mice are used. Animals must be kept at a temperature below 76°F. When animals are raised in an environment warmer than 76°F. a high incidence of "false positive" ovarian hyperemia reactions occurs, even if the animals are kept at below 76°F. for one week before use. These reactions apparently are the result of the high temperature, which causes a generalized vasodilatation, including the vessels of the ovaries.

The size and age of the animals used are factors influencing the incidence of false positive reactions. Animals over 12 to 13 grams in weight and older than 26 days tend to have a higher incidence of "false positive" reactions. Animals which are sick or are extremely small may have decreased sensitivity. We use only animals weighing from 8 to 13 grams, which are healthy and under 26 days of age. These animals are kept in an air-conditioned room at a temperautre between 72° and 76°F.

Specificity of the hyperemia reaction:

The hyperemia reaction in the mouse has been caused by the following materials given subcutaneously: sheep pituitary gonadotropin,[3] purified prolactin,[3] and extracts of urine from normal adult males and females and from castrate males. No reaction was caused by subcutaneous or intraperitoneal injection of extracts of urine from menopausal women (with one exception, when a small amount of hyperemia-producing material was found) and of extracts of urine from 3 patients with pituitary failure resulting from intracranial neoplasms. Intraperitoneal and subcutaneous injection of turpentine, casein (20 per cent) and glucose (25 per cent) in doses as large as could be tolerated by the recipient animals caused no ovarian hyperemia.

Route of administration and time of reaction:

The subcutaneous injection of gonadotropin extract in a single dose produces an ovarian response of magnitude equal to the response following intraperitoneal or subcutaneous injection of divided doses. Intraperitoneal

[3] Supplied through the courtesy of Dr. E. C. Reifenstein, Jr., of Ayerst McKenna & Harrison, Ltd.

injection did not increase the specificity of this reaction for luteinizing hormone in a manner such as occurs when other end-points are used (15) and the greater toxicity of the extract when given by this route presented a disadvantage outweighing any possible advantage. Autopsy at seven hours after injection resulted in greater sensitivity than did autopsy at two, four or twenty-four hours after injection.

RESULTS

Normal adults:

The gonadotropin excretion of a normal adult woman during a menstrual cycle as measured by the uterine weight and ovarian hyperemia techniques is depicted in Figure 1. Each point represents the value of half of the gonadotropin contained in a 48-hour urine collection. The necessity

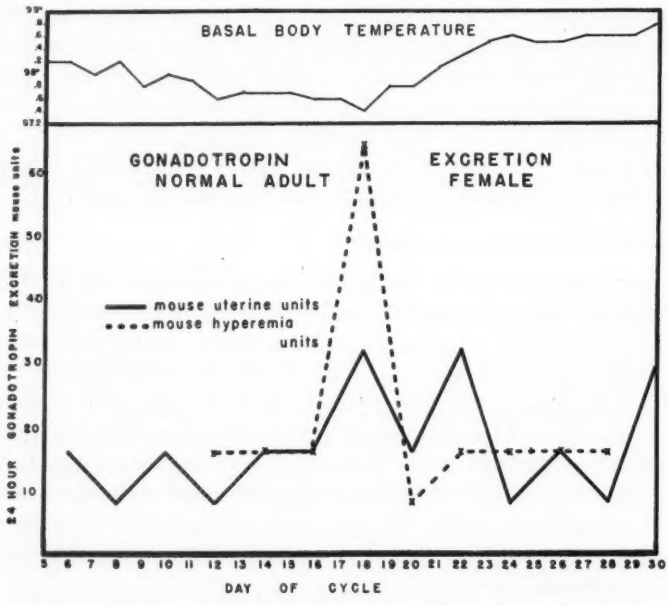

FIG. 1. Gonadotropin excretion of a cyclically menstruating woman throughout one menstrual cycle.

of pooling 48-hour extracts has undoubtedly resulted in decreasing the differences in levels of gonadotropin which might have appeared if daily measurements had been made. Study of two other complete cycles, one in the same subject, the other in a second normal woman, showed the same general pattern of gonadotropin excretion. A rise in total gonadotropin and

a proportionately much greater rise in hyperemia-producing material occurred just before the change in basal body temperature considered indicative of ovulation. In Figure 2 are shown representative data concerning the excretion of hyperemia-producing material of other normal women.

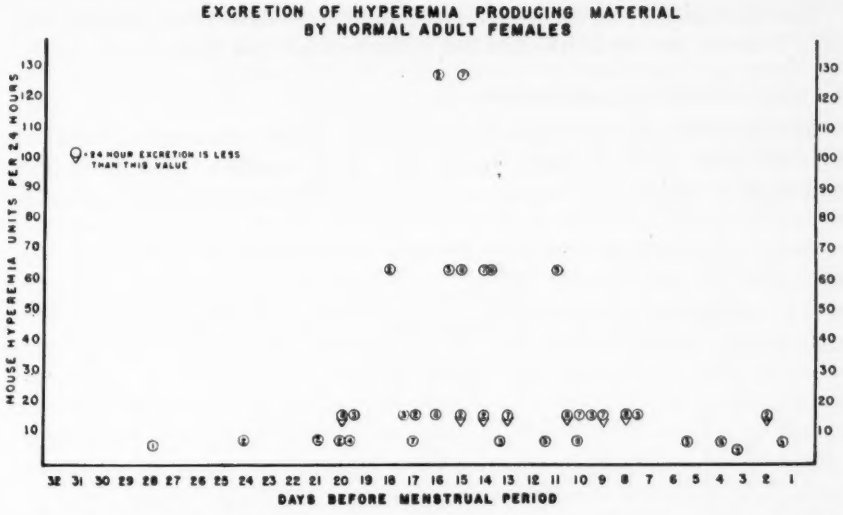
EXCRETION OF HYPEREMIA PRODUCING MATERIAL
BY NORMAL ADULT FEMALES
MOUSE HYPEREMIA UNITS PER 24 HOURS
24 HOUR EXCRETION IS LESS THAN THIS VALUE
DAYS BEFORE MENSTRUAL PERIOD

FIG. 2. Excretion of hyperemia-producing material by normal women. Each circle represents the excretion during one 24-hour period. The number within the circle identifies the subject.

Hyperemia-producing material is almost always detectable in the urine of normal women, with large amounts being present at the time of ovulation. The material is less frequently found in the urine of normal males, but is often detectable. Values for normal men are shown in Table 1. The

TABLE 1. GONADOTROPIN EXCRETION OF NORMAL ADULT MALES

Subject	Mouse uterine units per 24 hours	Mouse hyperemia units per 24 hours
1	<8	16
2	<8	< 4
3		<16
4	16	8
5		16
6	8	<16
7		<16
8	<8	<16
9	<8	<16

average values of hyperemia-producing material for men are lower than for women. No postcoital increase in hyperemia-producing material in either sex, such as has been reported by Farris (16), has been found.

Normal children:

Two normal boys and 2 normal girls, all in their sixth year, excreted less than 4 m.u.u. per 24 hours and less than 4 m.h.u. per 24 hours.

Hypergonadotropic hypogonadism:

Female: Five patients with ovarian failure either as a result of surgical castration or of physiologic menopause were studied. Uterine weight and ovarian hyperemia values are shown in Table 2. Although gonado-

TABLE 2. GONADOTROPIN EXCRETION OF CASTRATE FEMALES

Subject	Mouse uterine units per 24 hours	Mouse hyperemia units per 24 hours
	Untreated	
1	128	<6
2	128	<4
3	96	<8
4	96	<8
5	32	<5
6	64	16
	Estrogen treated	
7	<8	128
8	<8	256
6		64

tropin excretion as estimated in mouse uterine units was usually high, hyperemia-producing material was low or absent in all patients studied. Three other patients, surgically castrated several years before, were receiving sufficient estrogen at the time of study to produce a marked estrogen effect in the vaginal smear. These patients excreted large amounts of hyperemia-producing material and small amounts of total gonadotropin.

DISCUSSION

A considerable body of evidence indicates that the substance measured by the uterine weight method of gonadotropin assay is different from the material which produces ovarian hyperemia. The uterine weight increase is the result of the total gonadotropin activity present in the urine extract (7, 8). The ovarian hyperemia reaction depends on luteinizing hormone or luteotropin (10, 12, 13). Although the presence of follicle-stimulat-

ing hormone slightly augments the ovarian hyperemia-producing activity of luteinizing hormone, the degree of augmentation is not great (13). For clinical assays this augmentation is disregarded. In our studies, the uterine weight method of assay is considered as a measure of the total gonadotropin present in the extract, and the hyperemia-producing material is considered to be either luteinizing hormone or luteotropin. No method is available for differentiation between luteinizing hormone and luteotropin, so that it is impossible to decide what portion of the hyperemia-producing activity of an extract depends on each of these hormones.

If the hyperemia-producing material found in urine represents luteinizing hormone, its excretion by the normal adult is to be expected. The presence of luteinizing hormone is required for the secretion of gonadal steroid hormones (7). The normal adult is never completely without gonadal hormones and, therefore, some small amount of luteinizing hormone must be present at all times. In the female, the increase in hyperemia-producing material precedes ovulation and probably represents the luteinizing hormone which causes ovulation. We have found a high peak of excretion at the time of ovulation when urine concentrates are used. We have not been able to confirm the observation of Farris (11) that sufficient hyperemia-producing material is excreted before ovulation to produce a positive response when unconcentrated urine is injected.

Levin and Tyndale (1) and Leathem and Levin (17) have likened the gonadotropin found in urine of normal males to that found in the urine of castrate females, reporting that the urine of males has a high content of follicle-stimulating activity and a small amount of luteinizing activity. In our study, the average of values of hyperemia-producing material in urine extracts was less in males than in normal females, but was greater than for castrate females. The average total urinary gonadotropin was somewhat less in normal males than in normal women and was much less than the total gonadotropin excretion of castrate women.

The castrate women included in this study excreted large amounts of total gonadotropin and small amounts of hyperemia-producing material. In this type of patient the total gonadotropic activity consists principally of follicle-stimulating hormone (1, 2, 18, 19, 20, 21). The low values for luteinizing hormone determined by the hyperemia method are in agreement with the low values found by other means for detecting luteinizing activity. Although the luteinizing activity is low, definite though slight hyperemia-producing effects are sometimes obtained with urine from castrates. Other laboratories have also reported data indicating that the gonadotropin in the urine of castrates is not a pure follicle-stimulating hormone but also contains small amounts of luteinizing hormone (22). Preliminary studies, as indicated in Table 2, suggest that the urine of

castrate women who are receiving sufficient estrogen to produce a definite vaginal smear effect, contains hyperemia-producing material in amounts as great or greater than is found at the time of ovulation in the urine of normal women. Further studies of the urinary excretion of gonadotropin by castrate males and females and of the effects of estrogen on gonadotropin excretion will be published in a separate report.

SUMMARY

1. A method is described for measuring the urinary excretion of material which is capable of causing ovarian hyperemia when injected into the immature mouse. Mouse uterine weight assays were also performed on the same specimens.

2. The assay which measures the amount of ovarian hyperemia-producing material is considered to be an estimation of luteinizing hormone or luteotropin excretion.

3. The assay which utilizes the uterine weight increase as an end-point is considered to be an estimation of total gonadotropin excretion.

4. The normal woman excretes at all times material which causes an increase in the uterine weight of the mouse and which produces an ovarian hyperemia. Ovulation is preceded by a moderate increase in total gonadotropin and by a proportionately much greater increase in hyperemia-producing material.

5. The normal male excretes less of the hyperemia-producing material than is excreted by the normal female.

6. The castrate female excretes large amounts of total gonadotropin and very small amounts of the hyperemia-producing material. Administration of estrogen in quantities sufficient to cause a considerable estrogen response of the vaginal smear is followed by excretion of small amounts of total gonadotropin and large amounts of hyeremia-producing material.

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THE EXCRETION OF NEUTRAL LIPID-SOLUBLE REDUCING SUBSTANCES BY INFANTS*

CHARLES F. MATSON, M.S. AND BERNARD B. LONGWELL, PH.D.

From the Department of Biochemistry, University of Colorado School of Medicine, Denver, Colorado

THE development of chemical methods (1, 2, 3) for the measurement of adrenocortical steroids in urine has made possible the evaluation of the level of excretion of these substances in normal individuals and in relation to diseases known to involve the adrenal cortex. A considerable amount of data is now available concerning the normal level of excretion of adrenocortical steroids by adults. Little has been done toward the determination of their elimination by infants. Knowledge of the excretory level of these compounds during the first few weeks of life may aid in explaining the function of the human fetal adrenal cortex. In order to estimate the level of excretion of adrenocortical steroids in normal infants and in the hope that these data might help toward the evaluation of the function of the fetal cortex, the amount of neutral lipid-soluble reducing substances excreted in the urine of premature and full-term infants was determined during the period when involution of the fetal cortex is known to occur.

METHODS

Twenty-four hour urine specimens were collected from babies in the Premature Center and the Pediatric Ward of the Colorado General Hospital.[1] The urine was collected without preservative. It was stored in the refrigerator and was analyzed within twenty-four hours after collection. The determinations of neutral lipid-soluble reducing substances[2] were done by the method of Heard, Sobel and Venning (2) and the results are expressed in terms of desoxycorticosterone[3] as a standard. Analyses per-

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[1] We gratefully acknowledge the cooperation of Dr. Clifton D. Govan and staff members of the Department of Pediatrics who collected the urine specimens. The method of Hoag (4) was used for the urine collections.

[2] The method used is not specific enough to identify these substances unequivocally as adrenal cortical compounds.

[3] We wish to express our thanks to Dr. C. R. Scholz of Ciba Pharmaceutical Products, Inc., who furnished desoxycorticosterone.

formed on urine from adults gave results between 1.04 and 2.54 mg. per 24 hours, in comparison to the range of 1.1 to 2.1 mg. reported by Heard, Sobel and Venning (2). Recovery experiments with desoxycorticosterone were satisfactory.

RESULTS

All of the babies examined were males. Twelve determinations on urine specimens from 8 premature infants and 5 determinations on specimens from 5 full-term infants were made. The results are recorded in Table 1.

TABLE 1. THE URINARY EXCRETION OF LIPID-SOLUBLE REDUCING SUBSTANCES PER 24 HOURS BY INFANTS*

	Subject	Birth weight	Weight at time of collection of urine	Age at time of collection of urine	Urine volume per 24 hours	Lipid-soluble reducing substance per 24 hours
		Gm.	Gm.	days	ml.	mg.
Premature Infants	1.	2,119	2,041	3	49	0.17
			2,112	9	87	0.29
			2,182	15	57	0.30
	2.	2,140	2,098	13	50	0.20
			2,404	20	116	0.34
	3.	1,911	2,098	9	130	0.38
	4.	2,240	2,249	12	350	0.22
	5.	2,232	2,183	15	113	0.27
	6.	2,017	2,313	25	†	0.27
			2,495	28	211	0.36
	7.	1,903	2,580	27	112	0.20
	8.	1,389	2,108	33	147	0.22
Full-term Infants	9.	3,048	2,835	5	155	0.29
	10.	3,300	3,473	5	105	0.26
	11.	3,600	3,459	4	320	0.62
	12.	3,147	3,090	6	164	0.31
	13.	3,441		17	232	0.28

* Referred to desoxycorticosterone as a standard.

† Part of sample lost. Value given is 0.27 mg. per 150 ml.

The amounts of lipid-soluble reducing substances excreted by the premature infants varied from 0.17 mg. to 0.38 mg. per 24 hours. The full-term infants excreted a minimum of 0.26 mg. and a maximum of 0.62 mg. per 24 hours.

DISCUSSION

The results show that measurable amounts of lipid-soluble reducing substances are excreted by both premature and full-term infants. Day (5)

has reported values for the excretion of these substances by infants. The excretory levels varied from 0 (no reduction) to 0.2 mg. per 24 hours as measured by the method of Talbot, Saltzman, Wixom and Wolfe (1). These values are considerably lower than those reported here, but the method used by Day characteristically gives lower results than does the method of Heard, Sobel and Venning (2). Venning and Kazmin (6) were unable to detect any glycogenic activity in the urine of newborn male infants during the first four days of life.

In the present work there was no instance in which the urine failed to contain lipid-soluble reducing substances. In fact, the highest value observed, 0.62 mg., was excreted by a full-term infant 4 days of age. The smallest quantity, 0.17 mg., was excreted by a premature infant 3 days of age. The latter value is questionable because the small quantity of urine prevented duplicate determinations. Although the amount of data is too small to be certain, there appears to be no correlation between the age of the infant and the quantity of lipid-soluble reducing substances excreted. Neither does the amount excreted seem to depend upon whether or not the infant was born prematurely.

The babies in this study excreted much less of the lipid-soluble reducing substances than do adults. On a body weight basis, however, adults excrete approximately 0.02 mg. per Kg. per day, whereas these infants excreted approximately 0.12 mg. per Kg. per day. This would seem to indicate that the gland at this age is working at a higher level than that of the adult, although it is possible that the substances measured by this procedure may originate, in part, in locations other than the adrenal cortex. As Day (5) has pointed out, if one compares the level of excretion to the weight of the adrenal gland, this difference between the infant and the adult probably would disappear. However, Sayers and Sayers (7) emphasize that the level of secretion of the adrenal cortex is determined, in part at least, by the demand for the hormones that exists in the tissues. If this is the case, the expression of the level of excretion of the adrenocortical steroids in terms of body weight is perhaps a good method of comparison. On this basis our data indicate a greater cortical secretion in infants than in adults.

The question of the function of the human fetal adrenal cortex remains an enigma. The results herein reported might be taken to indicate that the fetal cortex produces substances similar to those produced by the adult cortex, and a larger quantity of them. On the other hand, the apparently greater secretion might be the result of the loss of stored materials occasioned by the involution of the fetal cortex. Certainly these data do not give any precise information concerning the function of this interesting organ.

SUMMARY

The lipid-soluble reducing substances have been determined in the urine of premature and full-term infants. The total quantity of material excreted was low but, per unit of body weight, was higher than that excreted by adults. The possible bearing of the results on the problem of the function of the fetal adrenal cortex is discussed.

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Letter to the Editor

To the Editor:

HYPERSENSITIVITY TO PITRESSIN

I SHOULD like to take this opportunity to write to you concerning the development of hypersensitivity to pitressin (beta-hypophamine) in the treatment of idiopathic (primary) diabetes insipidus. Such a situation occurred in a 33-year-old female who was receiving pitressin therapy for known diabetes insipidus.

This patient had been given one or two nasal tampons saturated with 1 to 2 cc. of pitressin in aqueous solution as required to control the diuresis for approximately two and one-half years. Gradual failure to influence the diuresis necessitated discontinuation of this form of therapy, and the intramuscular injection of 1 cc. of pitressin tannate in oil daily was instituted. After one month of using this route, erythematous areas appeared over the injection sites. On several occasions there occurred malaise, general aching, and a feeling of stiffness in the hands, feet and lips. There was one episode with some vomiting and tetany with generalized clonic spasms and marked carpopedal spasm.

An intracutaneous test with an aqueous solution of pitressin produced a pruritic, erythematous, elevated wheal 4.5 cm. in diameter.

The administration of 400 to 500 mg. of benadryl (diphenhydramine hydrochloride) daily has prevented local and systemic symptoms of allergy and has permitted the administration of the quantity of pitressin tannate required to control the diuresis and polydipsia. However, pressure urticaria at sites where constricting clothing presses against the body is a persistent, troublesome symptom.

When there has been a deficient intake of the antihistamine drug, without change in the amount of pitressin tannate, systemic symptoms have been presaged by alkalinity of the urine. Fifteen to 30 grains of ammonium chloride gave the urine a neutral reaction, abolished the malaise, aching, and stiffness; and nullified the antidiuretic action of the pitressin tannate.

When the pitressin tannate was given with a single, simultaneous dose of benadryl (100 mg.) and blood studies were done ten hours later (the usual time interval between giving the pitressin and the onset of untoward symptoms), the levels of calcium, phosphorus, albumin, globulin, total proteins and A/G ratio were all within normal limits.

The appearance of bizarre symptoms in individuals receiving continuous pitressin therapy should make one alert to the possibility of the development of hypersensitivity to this substance.

Manchester Road, R.D. 3,
Poughkeepsie, N. Y.

John Brice Plass, M.D.
January 4, 1949.

THE ASSOCIATION
FOR THE STUDY OF
INTERNAL SECRETIONS

ABSTRACTS OF PAPERS PRESENTED AT THE THIRTY-FIRST ANNUAL MEETING

FRIDAY AND SATURDAY, JUNE 3 AND 4, 1949

Haddon Hall

ATLANTIC CITY, NEW JERSEY

Scientific Sessions in the Viking Room

(Abstracts arranged according to number on printed program.)

FRIDAY, JUNE 3, 1949

9:15 A.M.—C. N. H. LONG, PRESIDING

1. Piperido-methyl-benzodioxane (933-F): Some Pharmacological and Experimental Observations.

Evan Calkins (by invitation), George W. Dana (by invitation), J. C. Seed (by invitation) and John Eager Howard, M.D.

From the Department of Medicine, The Johns Hopkins University and the Army Chemical Center, Edgewood, Maryland.

Piperido-methyl-benzodioxane (933-F) reversibly opposes the pressor and most of the other effects of epinephrine and allied compounds by competition for the epinephrine-specific receptor substance. Comparison of the formulae of the benzodioxanes, yohimbine, and the other reversible epinephrine inhibitors indicates that all these substances have the C-O-C-C-N group in common. Dibenamine, however, which irreversibly blocks the pressor action of epinephrine, lacks the oxygen atom in this group. It presumably combines chemically with the receptor substance at this point.

Sharp reduction in blood pressure following the intravenous injection of approximately 20 mg. of 933-F has been reported in 4 cases of pheochromocytoma. The present authors find similar responses, not only in a patient with pheochromocytoma, but also in a patient with neuroblastoma. Both patients were relieved of their hypertension following surgical removal of the tumors, although in the second case the hypertension returned with the development of liver metastases.

The blood pressure responses and side effects following administration of 933-F to over 100 patients with hypertension from other causes are described. In none of these was there a significant fall in blood pressure or any other ill effects. 933-F appears to provide a safe and effective clinical test for an excess of circulating epinephrine or epinephrine-like substance.

2. Nor-Epinephrine in Adrenal Medulla.

Marcel Goldenberg and Mogens Faber (introduced by R. F. Loeb).

From the Departments of Medicine and Biochemistry, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York, N. Y.

Studies on the hemodynamics of epinephrine and nor-epinephrine and its possible relation to the problem of hypertension have raised the question as to whether nor-epinephrine is present in the normal adrenal medulla and in chromaffin tissue tumors (pheochromocytomas).

Since bioassay of small amounts of nor-epinephrine in mixtures containing large amounts of epinephrine is not feasible, chemical methods were used to show the presence of nor-epinephrine in extracts of the adrenal medulla of cattle.

A good separation of nor-epinephrine from epinephrine can be obtained by paper chromatography using phenol as solvent (James). By modifying James' method, a sensitivity as low as 2 micro g. could be obtained and a quantitative estimation by planimetric measurement of the spot areas was possible.

Extracts of the adrenal medulla of cattle (epinephrine U.S.P. reference standard) were found to contain up to 18 per cent nor-epinephrine.

The epinephrine fractions from 3 chromaffin tumors were examined and found to contain 50 to 90 per cent nor-epinephrine.

These findings are of interest since epinephrine and nor-epinephrine differ significantly in their pharmacologic actions. Recent hemodynamic investigations in man using the method of right heart catheterization have shown that epinephrine within a physiologic range acts as an overall vasodilator and causes hypertension only by increase of cardiac output. Nor-epinephrine, on the other hand, acts as overall vasoconstrictor with no change or slight decrease of cardiac output. These two agents also differ in their actions on metabolism; *e.g.*, the hyperglycemic action of nor-epinephrine is much less marked than that of epinephrine (ratio 1:8).

3. Studies on an Anti-Diuretic, Non Chloruretic Substance Extracted from Urines of Normal and Cirrhotic Subjects.*

Elaine P. Ralli, Stephen Leslie (by invitation), George H. Stueck, Jr. (by invitation), Mary E. Dumm and Bertram Laken (by invitation).

From The Laboratories of the Department of Medicine, New York University College of Medicine.

The method of extracting the anti-diuretic substance from urine has been modified as follows: 24-hour urine samples divided in aliquots of 200 cc. contained in Visking cellulose casing (36/32 Nojax) were dialyzed for two hours against 3 changes of distilled water. The N and Cl concentration of the urine decreased during dialysis to less than 10% of the original level. The urines were concentrated about 25 times, pooled, and chromatographed, using a permutit column, which was eluted first with distilled water and then with 5% NaCl in 1 M acetic acid.

Na, Cl, N and arginine were determined in the eluates. Anti-diuretic activity was estimated by the bioassay method of Burn. No significant anti-diuretic activity was present in the distilled water eluates. However, large amounts of A. D. S. were found in the NaCl-HAc eluates.

The urine chlorides were determined during the period of bioassay and compared to

* This research was aided by a grant from the United States Public Health Service, Research Grants Division.

the urine chlorides following the injection of commercial pitressin in doses of equivalent anti-diuretic activity. Although the eluates had a marked anti-diuretic effect, the chloride concentration was not increased (16–21 mEq./*L.*), whereas commercial pitressin had a marked chloruetic effect (42–112 mEq./*L.*).

4. A Method for the Assay of Prolactin in Human Urine.*

Richard L. Coppedge (by invitation) and Albert Segaloff.

From the Departments of Physiology and Medicine of Tulane University and The Alton Ochsner Medical Foundation of New Orleans, Louisiana.

Prolactin is extracted from 24-hour urine specimens by acid-alcohol precipitation and dialysis against 0.5% saline. Assay by the local method of Lyons, as modified by Hall, is not entirely satisfactory because the prolactin response is often obscured by a non-specific inflammatory reaction. Systemic assay, employing our intravenous method, gives results which correlate satisfactorily with those of local assay. In both methods, pigeons are injected daily for four days and sacrificed on the fifth day and the crop observed.

Local assays on 4 normal women revealed a urinary prolactin excretion (as I.U./24 hrs.) in the first subject of <25; in a second, on 3 specimens, (1) <50 and 20, (2) 25 and 20, (3) >25 <50; in a third, on five specimens, (1) 100, (2) 100, (3) 60, (4) <50, (5) >50 <100 (87 by intravenous method); and in a fourth, >25 <100. One normal male showed values of 50 and 100, and another >50 <100.

Local assay of urines with added prolactin reveal values proportional to the amount of hormone added.

Assays have been made in patients with breast carcinoma, chronic cystic mastitis, fibromyomata uteri, endometriosis, precocious puberty, pituitary tumor, Cushing's syndrome (adrenal carcinoma) and pre- and post-partum. As yet, the level of prolactin excretion cannot be definitely correlated with the pathology or with other endocrine assays.

5. Thyrotropic and Thyroid Hormone Assay of Normal and Pathologic Human Sera in the Stasis Tadpole.

S. A. D'Angelo, A. S. Gordon, K. E. Paschkis and A. Cantarow.

From the Department of Biology, Washington Square College of Arts and Science, New York University and Jefferson Medical College, Philadelphia, Pa.

It has been demonstrated previously that the stasis (starved, non-metamorphosing) tadpole is a sensitive test object for the detection of thyrotropic hormone and thyroxine, and that the resulting metamorphosis with either agent can be differentiated by direct examination of the thyroid in the test animal (D'Angelo, Gordon, and Charipper, *Endocrinology* **31**: 217, 1942). The present experiments indicate that the method can be used to determine the status of thyroid-thyrotropic balance in the blood of normal individuals and those with endocrine disorders. Five to seven injections of 0.5 cc. of untreated serum, given on alternate days, advanced metamorphosis in the test animal. Correlation of the developmental changes with presence or absence of thyroid gland activation revealed that the thyroid-TSH equilibrium in the blood is shifted in endocrinopathies. In hypothyroidism (2 cases) serum was high in TSH but low in thyroid hormone. In thyrotoxic patients (2 cases) thyroid hormone was high with no TSH detectable. TSH was high in the blood of euthyroid individuals with moderate or severe ophthalmopathy (2 cases). Thyroid medication reversed the thyroid-TSH balance in one patient but failed in an-

* This work was supported in part by a grant from the National Institute of Health and in part by a grant from the National Research Council, Committee on Endocrinology.

other although the BMR was normalized. Thyroid hormone and TSH were present in excess in acromegaly. Normal sera possessed thyroid hormone activity with either minimal or no TSH present.

The results show that fluctuations in the thyroid-thyrotropic hormone balance characterize the circulating fluids in thyroid and pituitary gland disturbances, and that these can be detected in the tadpole with as little as 0.35 cc. of untreated serum.

6. Estimation of Urinary Gonadotropin of the Nonpregnant Human by the Mouse Uterine Weight and Ovarian Hyperemia Responses.

Charles W. Lloyd, Muriel Morley (by invitation), Kathryn Morrow (by invitation), Julia Lobotsky (by invitation) and Edward C. Hughes (by invitation).

From Syracuse University College of Medicine, Syracuse 10, New York.

A study has been made of urinary excretion of material capable of causing ovarian hyperemia when injected into immature mice. Mouse uterine weight assays were performed on the same specimens. The material producing ovarian hyperemia is considered to be either luteinizing hormone or luteotropin. The uterine weight assay is thought to estimate total gonadotropin excreted.

Normal women excrete at all times uterine weight-increasing and ovarian hyperemia-producing materials, with a moderate increase in total gonadotropin and a proportionately much greater increase in hyperemia-producing material at ovulation. Less hyperemia-producing material is excreted by normal men.

Female castrates excrete large amounts of total gonadotropin and small amounts of hyperemia-producing material. Estrogen therapy causes a reversion toward the normal pattern with production of large amounts of hyperemia-producing material.

The urine of castrate males contains large amounts of total gonadotropin and hyperemia-producing material. Androgen therapy causes decrease of hyperemia-producing material to normal levels and estrogen therapy does not increase excretion of this substance. A patient with Klinefelter's syndrome had high total gonadotropins but normal hyperemia-producing material.

It is suggested that fundamental differences exist in function and secretory control between the anterior pituitaries of adult human males and females.

7. Further Studies of Antigonadotropin Formation in Man.

James H. Leathem and A. E. Rakoff.

From the Bureau of Biological Research, Rutgers University, New Brunswick, N. J. and the Department of Obstetrics and Gynecology and the Endocrine Division, Jefferson Medical College and Hospital, Philadelphia.

Of 42 patients treated with a combination of sheep anterior pituitary and chorionic gonadotropin (Synapoidin), 5 developed antihormones. All were patients with functional menstrual disorders. The antiserum was nonspecific in that it antagonized the gonadotropic action of chorionic gonadotropin and pregnant mare serum.

Equine pituitary gonadotropin (Squibb) studies have been extended to 26 patients, 12 of whom developed antihormones. The gonadotropin was usually administered in 50 unit dosages two to three times weekly but for only two weeks of each month in females. Unlike the antisera obtained from rabbits treated with equine pituitary, the antigonadotropic action of human serum is not specific. The human anti-equine pituitary sera counteracted the gonadotropic action of pregnant mare serum in 8 of 8 cases, of chorionic gonadotropin in 7 of 8 cases and of sheep pituitary plus chorionic gonadotropin in 2 of 6 cases. The action of human pituitary material was not influenced. In 3 cases no

anti-equine pituitary action was obtained but the same sera nullified the gonad stimulating action of pregnant mare serum.

8. The Evaluation of the Use of Anterior Pituitary Extract in the Treatment of Pituitary Dwarfism.

Joseph C. Edwards, Cecil M. Charles (by invitation) and Cyril M. MacBryde.

From the Department of Internal Medicine, Washington University School of Medicine, Saint Louis, Mo.

Eleven patients with pituitary dwarfism were studied and treated for periods ranging from one to six years. Control periods were employed before and at intervals during treatment with a pituitary extract containing the growth principle.

Method of Study:—After preliminary periods of study with accurate anthropometric measurements each of the 11 patients was given "Phyone" (Wilson) pituitary extract which contains both growth and thyrotropic factors. Graduated doses increasing up to 2 cc. thrice weekly were continued for two to six months with control intervals without treatment. Eight of the patients were given no thyroid, androgen or estrogen during their observations on pituitary extract treatment.

Results:—The height of only one of the 11 patients exceeded at the end of treatment the minimum normal for his age. The normal growth curves for boys and girls are shown in the same scale as the growth curves of this group, for comparison. Our patients failed to show an increase in stature or weight so definite that it could be attributed to the use of growth extract "Phyone" alone. In the treatment of dwarfism due to primary hypopituitarism, pituitary extract therapy alone is, at present, unsatisfactory. Failure to obtain a growth response in human beings has recently been demonstrated not only with the commercially available products but also with relatively large doses of the pure growth principle. The lack of response may result from interfering factors in patients or from inadequate amounts of the growth principle in the preparations administered.

9. On the Inability of Adrenocorticotropic Hormone or Epinephrine to Deplete the Ascorbic Acid of the Chick Adrenal.

Norman F. Boas (by invitation) and Joseph W. Jailer.

From the Endocrine Section of the Medical Services, and the Chemical Laboratory of The Mount Sinai Hospital, New York.

Long, Sayers, and their collaborators have demonstrated that the administration of ACTH results in a prompt fall in adrenal ascorbic acid and cholesterol. Exposure of animals to stress (*e.g.*, epinephrine, histamine, cold) also results in a decrease in adrenal ascorbic acid and cholesterol in the normal, but not in the hypophysectomized rat. In the light of these observations it has been proposed that the depletion of ascorbic acid is an index of the elaboration of adrenal corticosteroids. This fall in adrenal ascorbic acid has been demonstrated in the rat and guinea pig. We have observed that such is not the case in the chick. The adrenals of White Leghorn cockerels from 2 to 41 days of age were examined. Shortly after hatching, the ascorbic acid concentration of the adrenals was 117 mg. %. This increased to a maximum of 201 mg. % at 41 days, in roughly a straight line curve. Following the administration of 25 mg. of ACTH (33rd and 41st days) and epinephrine (14th, 18th, and 41st days), no alteration in the adrenal ascorbic acid content or concentration was produced. The implications of these observations are discussed.

10. Regulation of Pituitary Adrenocorticotropic Activity by Adrenal Cortical Hormones.

Chi-Ping Cheng (introduced by George Sayres).

From the Department of Pharmacology, University of Utah College of Medicine, Salt Lake City, Utah.

The content of adrenocorticotropic hormone (ACTH) in the pituitary of the rat is reduced to 20% of normal 24 hours after adrenalectomy. In animals with intact adrenals, nonspecific stress (scald, shamadrenalectomy) reduces the ACTH content of the pituitary by 50%. Pretreatment of adrenalectomized rats with desoxycorticosterone acetate (DCA) prevents the depletion which follows adrenalectomy (pituitary ACTH content, 43% of normal). The results lend additional support to the concept that pituitary adrenocorticotropic activity is regulated by the level of cortical steroids in the body fluids.

DCA in the adreno-demedullated rat produces a state of insulin hypertension which can be corrected by the concomitant administration of whole adrenal cortical extract. DCA elevates plasma sodium and decreases the excitability of the central nervous system; neither phenomenon occurs in rats given ACTH or whole extract of the adrenal cortex together with DCA. These results may be interpreted to mean that DCA inhibits the release of ACTH from the pituitary and depresses the endogenous production of cortical hormones; a steroid hormone imbalance develops which is characterized by an excess of DCA (salt regulating) and a deficiency of the carbohydrate regulating steroids.

11. Adequacy of Pituitary Adrenocorticotropic Function in Nutritional Deficiencies.

George Sayers.

From the Department of Pharmacology, University of Utah College of Medicine, Salt Lake City, Utah.

Two indices have been employed to appraise the functional activity of the pituitary-adrenal system of rats with chronic malnutrition (low-protein diet). Content of adrenocorticotropic hormone (ACTH) in the pituitary was measured by direct assay; rate of release of ACTH, by adrenal size and chemistry. Malnourished rats had approximately the same large pituitary store of ACTH as normal rats. The adrenals of the low-protein rats were smaller than normal and had a greater concentration of cholesterol, indicating reduced adrenal cortical activity, probably in response to reduced body need for cortical steroids. After scalding, the content of ACTH in the pituitary at 24 hours was reduced approximately 50% in both groups. The content was restored to normal in both groups at 48–72 hours, despite continued ACTH discharge. The capacity to discharge ACTH was not appreciably altered in the malnourished rats as judged by the increase in size of the adrenals and the reduction in adrenal cholesterol and ascorbic acid after scalding. Malnutrition affects the functional capacity of the pituitary-adrenal system only slightly; this is in contrast with its marked effect on the pituitary-gonad system.

12. Effects of Prolonged Adrenal Cortical Stimulation upon Free and Esterified Serum Cholesterol in Normal Men.

Jerome W. Conn, and William C. Vogel (by invitation).

Ann Arbor, Michigan.

In 3 separate experiments upon 2 normal men, 56 duplicate determinations of free and ester cholesterol (Shoenheimer-Sperry technic) were made upon the sera before, during and after a course (6–8 days) of large amounts of ACTH. The response was as follows: 1) A mild decrease of the ester fraction on the second day of ACTH with no

change of free cholesterol, 2) Beginning on the third day and becoming more intense with time, a sharp fall of the ester fraction (maximal fall 77 mg. % or 45% below baseline), 3) During this same period, a fall of free cholesterol (max. 17 mg. % or 28% below baseline), and 4) Upon cessation of ACTH, prompt rebound of free cholesterol to or above the baseline value (1–2 days), whereas it required six days of gradually increasing values before the ester fraction returned to baseline.

Although two less likely explanations exist, we interpret the data as indicating 1) that under forced stimulation of the adrenal cortex the blood constitutes an important source of material for synthesis of cortical steroids, 2) that esterified cholesterol is the immediate precursor of the steroidal hormones, and 3) that increased cholesterol synthesis can more easily keep pace with the increased need for this basic compound than can the process by which it is esterified.

13. The Possible Involvement of the Adrenal Cortex and Thyroid in Mobilization of Fat to the Liver.

Louis Levin.

From the Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York, N. Y.

Previous work has demonstrated that marked increase in the liver fat level may be induced in mice by administration of suitable pituitary extracts or by subjection to some, but not all, physical stresses. The present work consists of experiments attempting to elucidate the mechanism whereby liver fat is so rapidly mobilized.

Mice adequately treated with thiouracil respond to stress equally as well as do untreated mice, indicating that thyroidal activity is not necessary for liver fat mobilization. Crude pituitary extracts or pure adrenotropic hormone (Armour), though very effective in causing fat mobilization in intact or in shamadrenalectomized mice, are completely inactive in this respect in adrenalectomized mice. Adrenalectomy, without further treatment, results in slight loss of liver fat. Exposure of adrenalectomized mice to physical stress does cause a slight increase in liver fat content but the increase is of much smaller magnitude than that elicited by similar treatment of intact animals.

Administration of 17-hydroxycorticosterone, 11-dehydrocorticosterone and 11-desoxycorticosterone to adrenalectomized mice causes a slight increase in the reduced liver fat level but never enough to surpass that of untreated intact mice.

Bile secretion in mice is apparently completely inhibited by adrenalectomy but may be re-induced by treatment with suitable adrenocortical steroids.

2:00 P.M.—J. S. L. BROWNE, PRESIDING

14. Stimulation of Nitrogen Metabolism by Adrenal Cortical Extract During Insulin Hypoglycemia.

Frank L. Engle.

From the Department of Medicine, Duke University School of Medicine, Durham, N. C.

In previous studies it has been shown that the rate of urea formation in the nephrectomized rat could be used as a sensitive measure of the rate of protein catabolism during brief periods of observation. It was found that adrenal cortical extract (ACE) increased urea formation, this increase being prevented by intravenous glucose or amino acids two hours after the ACE but not by a fat emulsion or serum albumin similarly administered. Evidence was presented that the action of ACE was on protein breakdown rather than on deamination of amino acids. It was suggested that the availability of sources of

new carbohydrate, other than from the tissues or injected protein, determined whether protein catabolism would be stimulated by ACE. In the present investigation the need for new carbohydrate was accentuated by insulin hypoglycemia. It was found that insulin produced a small increase in urea formation in the first three hours and a larger increase in the second three hours after injection, but when preceded by two hours by 1.0 ml. of ACE per 100 grams body weight a three to four fold increase in urea formation occurred during the first three hours compared to animals receiving ACE or insulin alone. Prevention of insulin hypoglycemia by glucose completely abolished the increase in urea formation. The significance of these findings with respect to the mechanism of the protein catabolic action of the adrenal cortex and the possible role of stress per se in this phenomenon is considered.

15. Renal Function in Normal and Adrenalectomized Rats Following Saline or Adrenal Steroid Administration.

W. R. Boss (by invitation), James H. Birnie and Robert Gaunt.

From the Department of Zoology, Syracuse University, Syracuse, N. Y.

Studies of renal function in untreated adrenalectomized rats indicating that decreased glomerular filtration may contribute to the loss of water diuresis, but that an increase in tubular reabsorption is probably a more important factor, have been reported by the authors.

Creatinine clearance studies were conducted on female normal and adrenalectomized rats weighing approximately 175 grams. Sixty-three rats divided between the various groups studied were used in this investigation. The glomerular filtration rate (C-Cr) of normal rats given two doses of water (3 cc./100 sq. cm. body surface/dose) by stomach tube was .956 cc./100 Gm./minute and the urine flow (V) was .062 cc./100 Gm./minute. In rats adrenalectomized for seven days and maintained on a 1.0 per cent saline solution as drinking fluid the C-Cr was .738 and the V was .007. Rats adrenalectomized for seven days and maintained by daily subcutaneous injections of 0.5 mg. DCA had a C-Cr of .844 and a V of .018. These results indicate that while saline (as found by Lotspeich) or DCA will maintain essentially normal glomerular filtration in adrenalectomized rats the urine volume remains far below normal, presumably due to an elevated tubular reabsorption of water.

When 5.0 mg. of DCA was administered to intact rats the C-Cr was .900 and the V .077. Following the oral administration of 4 cc. of cortical extract (Upjohn) to intact rats the C-Cr was .993 and the V .076. The increase in the rate of urine flow without an accompanying rise in glomerular filtration in overdosage experiments is interpreted as indicating that these adrenal steroids cause a decrease in the tubular reabsorption of water. Our work with DCA confirms that of Winter and Ingram in dogs.

16. Adrenal Cortical Hormone in Blood.

K. E. Paschkis, A. Cantarow and D. Boyle (by invitation).

From Jefferson Medical College, Philadelphia, Pa.

Levels of adrenal cortical hormone were determined in the blood of dogs, using the mouse-liver-glycogen method of E. Venning. Small amounts of hormone were present in the arterial plasma of normal dogs. Injection of ACTH or of epinephrine caused a prompt rise of hormone levels.

A rise also occurred after subcutaneous injection of formalin (alarming stimulus). Intravenous injection of insulin was followed by an increase of cortical hormone levels, suggesting the participation of cortical hormone in counteracting insulin-induced hyperglycemia.

Attempts were made to determine whether the output of adrenal cortical hormone was mediated by epinephrine and could be prevented by adrenergic block. Injection of dibenamin was found to be an alarming stimulus, leading to an increase in blood levels of cortical hormone. Insulin as well as formalin induced a further rise.

17. Urinary Corticoids.

Eleanor H. Venning, M. P. Ripstein (by invitation) and V. E. Kazmin (by invitation).

From McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

In the following study a comparison of two chemical methods and a biologic assay for urinary corticoids has been made on urine collected from both normal and pathologic. The chemical methods used were based upon 1) the reduction of cuprous ion to cupric ion, and 2) the formation of formaldehyde following periodic acid oxidation. They were carried out on the same urinary fraction, the water soluble neutral corticoid fraction. The biologic assay which is dependent upon the deposition of glycogen in livers of adrenalectomized mice, was done on the total neutral corticoid fraction. The same standard, dehydrocorticosterone, was used for all assays.

In most of the normal cases, the reduction method gave higher values than the oxidation method. Greater variations were encountered with the reduction method than with the oxidation method.

In 9 cases, in which the biologic assay was below the normal range, with the exception of 2 cases, the values obtained by the chemical methods were also low. These patients suffered from pituitary tumors, Addison's disease, myxedema and malnutrition.

In 5 different lots of pooled urine collected postoperatively from patients, there was considerable disagreement between the biologic and chemical assay.

A study was also made on the effect of pH, and time, on the hydrolysis of urinary corticoid conjugates in urine.

18. Studies on the Interrelationship of Adrenal and Thyroid Function.

Robert S. Reiss, Peter H. Forsham (by invitation) and George W. Thorn.

From the Department of Medicine, Harvard Medical School and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Mass.

In 8 patients with Addison's disease the somwhat low gradients of radioactive iodine uptake, as measured by the method of Astwood and Stanley, following the oral administration of 100 mc. of I^{131}, were increased up to fourfold following the intramuscular injection of synthetic Compound E acetate (50 to 100 mg. per day) and Lipoadrenal Cortex (4 to 6 cc. per day). This effect was maximal after two to three days and decreased therafter. No such effects were observed in a case of panhypopituitarism.

The simultaneous administration of 0.3 mg. of epinephrine and I^{131} to 5 normal subjects led to an increased rate of iodine uptake by the thyroid, maximal after three to four hours coincidentally with the greatest depression in circulating eosinophils, known to be an indicator of adrenal cortical hormone secretion. Both responses were absent in a patient with panhypopituitarism who did show a positive response to thyrotropin and adrenocorticotropic hormone. Patients with Addison's disease failed to show either thyroid or adrenal cortical activation following 0.3 mg. of epinephrine subcutaneously.

It is suggested that certain adrenal cortical hormones facilitate the release and/or action of thyrotropin.

19. Clinical and Metabolic Changes in Addison's Disease Following the Administration of Compound E Acetate (11-dehydro, 17-hydroxy-corticosterone acetate).

P. H. Forsham (by invitation), L. L. Bennett (by invitation), M. Roche (by invitation), R. S. Reiss, A. Slessor (by invitation), E. B. Flink (by invitation) and G. W. Thorn.

From the Department of Medicine, Harvard Medical School and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Mass.

Fourteen patients with Addison's disease were treated with synthetic Compound E acetate, administered as a crystalline suspension in water, supersaturated suspension in oil and pellets. Striking clinical improvement followed the injection of 50 to 100 mg. of Compound E daily for two to six days and was sustained for several months in all 5 patients implanted with five to ten 50-mg. pellets. Absorption averaged 0.5 mg. per pellet per day. The most striking metabolic changes observed consisted of a return towards normal of the electroencephalogram, an increased uptake of radioactive iodine by the thyroid, a transient rise in fasting blood ketone bodies with an increased capacity to tolerate a 24-hour fast, and a small, but definite, retention of sodium and chloride in the absence of supplementary desoxycorticosterone treatment. On desoxycorticosterone a paradoxical loss of sodium and chloride occurred.

A small rise in blood sugar levels was accompanied by a decrease in urinary inorganic phosphorus and creatine excretion. Increase in uric acid excretion, with little or no increase in total nitrogen excretion occurred. Circulating eosinophils were depressed. Urinary 17-ketosteroids increased slightly; 11-oxysteroids did not.

20. Effect of a Single Dose of Desoxycorticosterone Acetate on Electrolyte Metabolism.

Paul Fourman* (by invitation), Edwin J. Kepler,** Edward C. Reifenstein, Jr. and Eleanor F. Dempsey (by invitation).

From the Department of Medicine, Harvard Medical School, the Medical Service of the Massachusetts General Hospital, Boston, Massachusetts; and the Department of Medicine, Mayo Clinic, Rochester, Minnesota.

A normal, 24-year-old, 70 Kg. man was put on a daily intake of 300 ml. of water, 3 Gm. of NaCl, 1 Gm. of KCl and 500 Gm. of glucose, given in 12 two-hourly aliquots. Four experiments were done, two control and two with 30 mg. of desoxycorticosterone acetate (DOCA) injected. Urine was collected in 9 two-hourly and then 4 six-hourly periods and the excretion of K, Na, Cl, P, Ca, N, NH_3 and titratable-acidity-minus-CO_2 was measured. For the 40-hour collection period the DOCA effect was as follows:

Metabolite	Effect	Amount
K	Loss	15.4 ± 3.5 mEq.
Na	Retention	50.5 ± 5.3 mEq.
Cl	Retention	39.2 ± 5.9 mEq.
Ca	Retention	44.9 ± 10.0 mEq.

There was no significant effect on N, P, NH_3, or titratable-acidity-minus-CO_2.

For the positive effects the following relations obtained:

mEq. Na retained

$$= (\text{mEq. Cl retained}) \times 1.00 \pm 0.03$$
$$+ (\text{mEq. K lost}) \times 0.76 \pm 0.08$$

* Rockefeller Traveling Fellow.
** Deceased.

mEq. Ca retained

$$= (\text{mEq. Cl retained}) \times 0.74 \pm 0.15$$
$$+ (\text{mEq. K lost}) \times 1.19 \pm 0.41$$

Sodium retention was limited by the amount available, and the portion of retained sodium that accompanied chloride was less than the amount of sodium normally found with chloride in extracellular fluid. Some sodium entered the cells, replacing potassium but less sodium entered than potassium left. Calcium retention was correlated with potassium loss independently of calcium retention due to increase of chloride space.

21. Changes in Urinary Steroids Produced by Sodium Deprivation and by Desoxycorticosterone Acetate Administration.

William H. Daughaday (by invitation) and Cyril M. MacBryde.

From the Department of Internal Medicine, Washington University Medical School, St. Louis, Mo.

If sodium reabsorption by the renal tubule is under intimate regulation by the adrenal cortex, it should be possible to detect changes in the excretion of adrenal metabolites under conditions of excess or deprivation of dietary sodium. Daily determinations of the urinary excretion of "cortin," (measured by liberation of formaldehyde from steroid residues, *J. Clin. Endocrinol.*, **8**: 166, 1948) and of 17-ketosteroids were done in 3 case studies over periods from one to two months with varied sodium intake and during the daily intramuscular administration of 10 and later 15 mg. of desoxycorticosterone acetate (DCA). Changes in steroid excretion were considered in relation to measured alterations in electrolyte and water balance. A woman with Cushing's syndrome showed elevated excretion of cortin and 17-ketosteroids with explosive and cyclic changes in the levels independent of sodium intake. Decreased excretion of 17-ketosteroids but not of cortin occurred during DCA administration. In 2 women with hypertension but without significant renal disease extreme sodium restriction did not result in increased urinary cortin. During DCA administration cortin excretion increased in one case, but was unchanged in the other.

Conclusion: Release of adrenocortical "salt hormone" is apparently independent of ACTH and 11-oxysteroids, and in these doses DCA does not inhibit ACTH liberation and cortin excretion.

22. The Evaluation of Adrenocortical Function by Ascertaining the Response to a Single Injection of Adrenocorticotropin.

H. W. McIntosh (by invitation), B. Singer (by invitation) and M. M. Hoffman.

From McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

Normal individuals, patients with Addison's disease and patients with pituitary tumors were studied in the following manner: control urine was collected from 12 midnight to 8 a.m. At this time an amount of ACTH equivalent to 21 mg. of the Armour standard was injected intramuscularly and urine collected during the subsequent six hours. The concentrations of uric acid, potassium, chloride, 17-ketosteroids and neutral reducing substances present in the control and post-injection urines were determined and the results expressed in relation to the creatinine concentration. The number of circulating eosinophils was determined before and four hours after the injection of ACTH.

In the 10 normal subjects there was, following the administration of ACTH, an increase in each of the urinary constituents studied of from 50 to 350%. The 5 patients

with Addison's disease showed little or no change in these constituents. It is concluded therefore that the changes which follow ACTH administration may serve as criteria for the evaluation of adrenocortical reserve.

Ten patients with pituitary tumors were similarly studied. Their response to ACTH was variable. In general they fell into two groups: those whose response was normal, and those whose response was abnormal by one or more of the criteria employed. The effect of the removal of the tumor on the response to ACTH in 4 patients is presented.

23. The Level of Circulating Eosinophils as an Indicator of Adrenal Cortical Adequacy Following Major Surgery.

Marcel Roche (by invitation), A. Gorman Hills (by invitation) and George W. Thorn.

From the Department of Medicine, Harvard Medical School and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Mass.

Earlier studies demonstrated the depression of circulating eosinophils following an increase in adrenal activity. In 43 patients, following major surgery, the level of eosinophils fell from normal (100 to 200/cu.mm.) to, or near, zero in all except 1 patient five to fifteen hours postoperatively. In this patient, fifteen hours following a colostomy for malignancy, the level of eosinophils was 296. At autopsy unsuspected bilateral adrenal tuberculosis was demonstrated.

Following operation, the eosinophils returned to or above preoperative levels within three to five days in most instances. At this time a fall in eosinophil level, in response to the administration of 25 mg. of standard adrenocorticotropic hormone intramuscularly, or 0.3 mg. of epinephrine subcutaneously, suggests that the postoperative rise in eosinophils is associated with a restoration of normal adrenal cortical reserve. It is suggested that, in patients with complicated postoperative convalescence, adrenal cortical insufficiency may be ruled out by finding an initial eosinopenia or observing a satisfactory response to ACTH or epinephrine.

24. Is the Protein Metabolic Abnormality of Cushing's Syndrome Catabolic or Anti-Anabolic?*

Sheldon Margen** (by invitation), Laurance W. Kinsell, Erin K. Flanagan (by invitation), Lila E. Suiter (by invitation) and Elliot Rapaport (by invitation), with the technical assistance of Vernon T. Thompson.

From the Division of Medicine, University of California Medical School and the Metabolic Research Unit, U. S. Naval Hospital-University of California Medical School, Oakland and San Francisco, California.

S^{35}-labeled-methionine in tracer dosage was administered intravenously to a 39-year-old female with active Cushing's disease, referable to a large tumor of the left adrenal gland. The incorporation of S^{35} in plasma protein, tissue protein, total urinary sulfate and urinary organic sulfur was determined during the period of study.

Results show that the initial incorporation of S^{35} into the plasma proteins occurs at a more rapid rate than in the normal. In the first 48 hours there is no greater urinary excretion of organic S^{35} (S^{35}-labeled-methionine) or $S^{35}O_4$ (catabolized labeled methio-

* This work was supported by grants from the Research Division, Bureau of Medicine and Surgery, U. S. Navy (BuMed #007046), and from the Office of Naval Research under a contract between the latter and the University of California.

** Senior Research Fellow, U. S. Public Health Service, 1947–48; and Schering Research Fellow in Endocrinology, 1948–49.

nine) than in the normal. These data therefore demonstrate that there is no primary anabolic defect in Cushing's syndrome; in fact there would appear to be a hyperanabolism which does not, however, compensate for the excessive catabolism that must occur. Incorporation of S^{35} into the protein of tissues obtained at the time of operation and autopsy is discussed.

25. Hypokalemic Alkalosis in Cushing's Syndrome. Observations on the Effects of Potassium Chloride and Testosterone Propionate Therapy.

Robert Teabeaut (by invitation), Frank L. Engel and Haywood M. Taylor (by invitation).

From the Departments of Medicine and Biochemistry, Duke University School of Medicine, Durham, N. C.

A 34-year-old man with Cushing's syndrome due to adrenal cortical carcinoma who showed striking electrolyte abnormalities (serum Na-143.5 milliequivalents/liter, K-2.56 milliequivalents/liter, Cl-83.3 milliequivalents/liter, and CO_2-41.0 milliequivalents/liter) was studied. He had diabetes mellitus (requiring insulin), hypertension, osteoporosis with collapsed vertebrae, and excreted 7.7 mg. of reducing steroids per day. The electrocardiogram showed characteristic changes of hypokaliemia. Intravenous administration of 28 grams of KCl (12.9 milliequivalents K^+/Kg./hour) restored the serum electrolyte pattern and electrocardiogram to normal in four hours. Twenty-one grams of KCl were retained during an eight hour period. Twenty-four hours later the serum potassium declined to 3.72 milliequivalents/liter, the CO_2 now being 31.5 milliequivalents/liter. In nine days the serum electrolytes had returned to their original levels. One hundred mg. of testosterone propionate daily for nine days resulted in a decline in the serum CO_2 from 37.8 to 21.6 milliequivalents/liter, serum potassium remaining low, *i.e.*, 3.0 milliequivalents/liter. These findings are consistent with the views of Darrow and Kepler that the alkalosis and hypokaliemia in this syndrome are secondary to intracelluiar potassium depletion. Testosterone presumably overcame the alkalosis by promoting intracellular potassium retention at the expense of extracellular potassium, the serum potassium thus remaining low.

26. The Mechanism of Action of Testosterone in the Therapy of Cushing's Syndrome.

Frederic C. Bartter, Anne P. Forbes, William M. Jefferies, Evelyn L. Carroll (by invitation) and Fuller Albright.

From the Massachusetts General Hospital, Boston, Massachusetts.

In previous reports it has been demonstrated that methyl testosterone and testosterone propionate have a beneficial effect in females with Cushing's syndrome. This involves: 1) a marked anabolism of protoplasm; 2) a decrease in 17-ketosteroids of endogenous origin; 3) a decrease in "11 oxysteroid" excretion.

A priori, testosterone could exert its effect by: a) inhibiting or neutralizing the action of adrenal cortical hormones on tissues, b) inhibiting the action of ACTH on the adrenal cortex, or c) inhibiting ACTH production.

Metabolic studies are presented to support the following arguments:

Testosterone produces anabolism of protoplasm in Addison's disease; thus, it must have an effect peripheral to the adrenal cortex.

Methyl testosterone, when administered simultaneously with ACTH, does not exert its full anabolic effect, nor prevent the rise in "11 oxysteroids," the fall in eosinophils, or the rise in 17-ketosteroids; thus it presumably does not inhibit the action of ACTH on the adrenal cortex.

Methyl testosterone does produce a fall in "11 oxysteroids" and in 17-ketosteroids in

certain females with Cushing's syndrome; thus it presumably can inhibit production of ACTH by the pituitary.

The data support the conclusion that testosterone has both a direct anabolic effect on tissue and an inhibitory effect on the pituitary in Cushing's syndrome.

SATURDAY, JUNE 4, 1949

9:00 A.M.—E. A. DOISY, PRESIDING

27. Metabolism and Distribution of Thiourea in the Rat as Measured with Radioactive Sulfur.

John Schulman, Jr. and Richard P. Keating (introduced by Rulon W. Rawson).

From the Los Alamos Scientific Laboratory, Los Alamos, New Mexico.

Thiourea labelled with S^{35} was injected intraperitoneally into young albino rats. The excretion and tissue distribution were measured at 6, 24 and 48 hours. Three animals were used at each time interval.

More than 98% of the radioactivity was excreted in the urine within 48 hours. Filter paper chromatography indicated that most of the thiourea was excreted unchanged. Chemical analysis showed that contrary to earlier reports 6% was excreted as inorganic sulfate and 6% as ethereal sulfate. Fecal excretion was slight. Traces of radioactive sulfur were found in the expired air.

The highest concentration of radioactive sulfur was found in the thyroid gland, reaching a maximum of 3.5% of the injected dose per gram of thyroid at 24 hours. The ratio of the concentration in the thyroid to the average concentration in the body rose from 11:1 at 6 hours to 37:1 at 24 hours and to 90:1 at 48 hours.

This rising ratio suggests that thiourea is bound in the thyroid gland.

28. The Tracer Technique with Radioiodine I^{131} as a Potential Substitute for the Basal Metabolic Rate Determination in Routine Clinical Practice.

Sidney C. Werner, Lawrence D. Goodwin and Edith H. Quimby.

From the Departments of Medicine and Radiology, College of Physicians and Surgeons, Columbia University and The Presbyterian Hospital in the City of New York.

A simple test to replace the basal metabolism has long been desired, in view of the inherent inaccuracies in the method and the effect of subjective influences upon the result. Accordingly, a large scale experiment has been instituted at the Presbyterian Hospital to compare the uptake of radioiodine with the basal metabolic rate in all ward patients on whom a determination of the latter has been requested.

The results in about 200 patients have been summarized: first, the number of patients in whom the basal rate determination could not be done or was apparently unreliable, and the number who could not be tested with radioiodine because of immediately preceding iodide or thyroid ingestion, etc.; second, the accuracy of the two procedures as a reflection of the final diagnosis; third, cost, speed of testing, technician capacity, and incidence of unexpected difficulties.

It is suggested that consideration should be given to the replacement of the basal metabolism as a screening procedure for thyroid dysfunction. The basal metabolic rate would be determined only if an abnormal tracer uptake were found, when the tracer value appeared discrepant, or when the course after treatment was being followed.

29. The Distribution and Metabolism of Circulating Testosterone.

C. D. West and L. T. Samuels.

From the Department of Biochemistry, College of Medicine, University of Utah, Salt Lake City 1, Utah.

Testosterone in solution in plasma was administered intravenously to rabbits, dogs and men. The rate of disappearance from the blood stream was determined, both in the intact individual and, in animals with the liver, kidney, or both liver and kidneys eliminated from the circulation. Elimination of either organ reduced the rate of disappearance while the elimination of both further reduced, but did not eliminate, the disappearance of the hormone.

Testosterone accumulated in greatest concentration in the fatty tissues, and next in the spleen, heart and skeletal muscle. All of these tissues had concentrations as high or higher than the blood at the time of sampling. Other tissues had lower levels; liver contained no significant amount.

In man, the 17-ketosteroid excretion curve was determined in relation to the blood testosterone and 17-ketosteroid levels. No significant amount of testosterone appeared in the urine even when blood levels were high. The effect on nitrogen metabolism is also discussed.

30. Pseudo-hypoparathyroidism: a Report of Two New Cases With Special Reference to the Epiphyseal Changes.

Harold Elrick (by invitation), Frederic C. Bartter, Adney Sutphin (by invitation) and Fuller Albright.

From the Massachusetts General Hospital, Boston, Massachusetts.

In 1942 (*Endocrinology* **30**: 922–932) the syndrome of pseudo-hypoparathyroidism was described. This syndrome resembles hypoparathyroidism with respect to the disorder of calcium and phosphorus metabolism but the immediate cause of the disorder is not a lack of hormone but a failure to respond to it. In addition, it is often associated with a characteristic facies and certain epiphyseal changes in the hands and feet.

Two new cases are reported. In one case x-rays from ages three to seventeen are available and demonstrate the evolution of the unusual epiphyseal changes. Parathyroid biopsy in one of the cases confirmed the presence of normal or hyperplastic parathyroid tissue.

Attention is called to a new physical sign characterized by peculiar movement of the lip during speaking.

31. Quantitative Measurements of the Growth of Axillary Hair as an Index of the Endocrine Status.

James B. Hamilton.

From the Department of Anatomy, Long Island College of Medicine, New York.

A continuously-growing secondary sex character, axillary hair, can be measured quantitatively. In 45 apparently normal men, 21 to 75 years of age, the curve of average values per decade of life parallels that for urinary steroids, although for individual men the parallel is far from exact. After the third decade of life the average curve of decline in weight of axillary hair in 420 subjects is straight in men. In women it is sigma-shaped and falls much more rapidly beginning with the fifth decade. The values are low in eunuchs, and are nil or less than 1 mg. in prepubertal castrates.

Weekly measurements of separately identified hairs confirm the findings obtained with

weighted shavings and emphasize the faster growth of large than of small hairs and the greater accuracy obtained with more refined measures than the gross weight.

Wide fluctuations occur at different periods of the year, even in the orchiectomized man injected daily with an unvarying amount of testosterone propionate.

32. The Effects of Testosterone Propionate on the Peripheral Blood and Bone Marrow of Women with Advanced Inoperable Carcinoma of the Breast. Preliminary Report.

Timothy R. Talbot, Jr.* (by invitation) and George C. Escher.*

Ten out of 70 patients under treatment with testosterone propionate for inoperable carcinoma of the breast showed an increase in hemoglobin, hematocrit and erythrocyte count: average values 49 to 58%; RBC 5.3 to 6.8 million per cu. mm. Six out of these 10 patients exhibited a moderate increase in cellularity of bone marrow or an increase of the erythroid-myeloid (E/M) ratio. Two of these patients had pulmonary metastases although neither showed an increased cellularity of the bone marrow or an increased E/M ratio.

No radiotherapy was given within a six month period prior to or during hormone therapy.

These data are offered as confirmatory evidence in the human female of changes that have been previously observed by others in animals. No explanation of this action of testosterone propionate can be suggested at this time.

33. Effects of Small Doses of Testosterone Propionate on Spermatogenesis.

Cleve Beller† (by invitation) and Henry H. Turner.

From the University of Oklahoma School of Medicine and Oklahoma Medical Research Foundation, Oklahoma City.

Studies are in progress to determine the effects of 5 mg. of testosterone propionate given parenterally twice weekly to subjects showing aspermia, oligospermia and normal sperm counts.

Subjects consists of a number of healthy medical students, and the series is being controlled by administration of sesame oil to a similar group. To date the normal subjects have shown no significant deviation in sperm count. The majority of those individuals with oligospermia are showing a significant increase in sperm count, while the single case of aspermia has shown no change.

The study is being extended to similar groups noting response to 2.5 and 1.25 mg. of testosterone propionate twice weekly for eight weeks.

34. Endocrine Factors in Gout: The Significance of Differences in Childhood and Adult Urate Metabolism.

William Q. Wolfson, David Krevsky, Rachmiel Levine (by invitation), Kinu Kadota (by invitation) and Clarence Cohn.

From the Departments of Biochemistry,‡ Metabolic and Endocrine Research, and Pediatric Research, Medical Research Institute, Michael Reese Hospital, Chicago 16, Illinois.

Children's urate metabolism has been found to differ from the normal adult's in a

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* Assistant, Sloan-Kettering Institute, Memorial Cancer Center, New York; Fellow American Cancer Society sponsored by the Committee on Growth of the National Research Council.

† Schering Research Fellow in Endocrinology 1948–1949.

‡ Aided by a grant from the Committee on Scientific Research of the American Medical Association.

direction generally opposite to the deviations in gout. Children's average plasma urate levels (3.3 mg.%) are lower even than normal women's (4.2 mg.%) and show no sex difference. Urine ratios of urate/preformed creatinine (0.68) and urate/total creatinine (0.52) and of urate clearance/endogenous creatinine clearance (0.15) are all higher in children than in adults. Others have ascribed the latter findings to relatively increased corticoid activity. Our data are consistent with the known high corticoid/androgen ratio in childhood. Cushing's syndrome, and sometimes acromegaly, show high corticoid/androgen ratios, and may show a childhood type of urate metabolism.

Androgenic regulation of urate metabolism is suggested by higher average plasma urate levels in men than women, both in normals and the gouty. Pre-gouty carriers of hereditary hyperuricemia have normal urate levels until puberty, if male, and until the menopause, if female (Smyth, Stecher and Wolfson: *Science* **108:** 514, 1948). Such observations imply androgenic control of hereditary hyperuricemia. A separate abnormal androgen has been postulated because a sex differential in plasma urate exists in gout. Very low 17-ketosteroid excretion in gout without hypogonadism and with unimpaired ability to convert administered testosterone to 17-ketosteroid also suggests an abnormal androgen (*J. Clin. Endocrinol.* **9:** 497 (June) 1949).

35. The Effect of Castration, of Unilateral Castration, and of Pregnancy in Unilaterally Castrate Rats on the Ovary Transplanted into the Spleen.

Gerson R. Biskind and Morton S. Biskind.

San Francisco and New York.

A regular sequence of events occurs after one ovary has been transplanted into the spleen of a castrate rat. After the initial inflammatory reaction has subsided there is a constant development of new follicles which luteinize. These corpora lutea do not involute and from them, after five months or more, a luteoma appears. Subsequently a granulosa cell tumor may replace the luteoma. If an ovary is transplanted into the spleen of a unilaterally castrate rat, the intrasplenic ovary atrophies. The growth potentialities of this atrophic intrasplenic ovary become evident as soon as the normal ovary is excised. The effect of pregnancy on the intrasplenic ovary of unilateral castrates is to produce active follicular growth without the formation of corpora lutea. Following termination of the pregnancy the intrasplenic ovary atrophies; however simultaneous termination of the pregnancy and castration produces active growth in the intrasplenic ovary. Certain aspects of the unusual hormonal stimulation in these experimental states are discussed.

36. The Occurrence of Conjugated Sulfates of Estrogens in Human Pregnancy Urine.

Herman Cohen (by invitation) and Robert W. Bates.

From the Endocrine Development Department, E. R. Squibb and Sons, New Brunswick, New Jersey.

The conjugation of estrogens in human urine with both sulfuric and glucuronic acid has been postulated, but no sulfate conjugated estrogen has been demonstrated.

Hydrolysis with the phenolsulfatase present in commercial enzyme preparations obtained from aspergillus oryzae was carried out directly on urine or on aqueous solutions prepared from butanol extracts from urine. It was demonstrated that a variable portion of the estrogens in human pregnancy urine is excreted as the sulfate ester. Among 7 different urine samples obtained from women in the seventh to ninth month of pregnancy, it was found that 5–89% of the estriol fraction and 8–100% of the estrone-estradiol fraction occurred conjugated with sulfuric acid.

Because of the specificity of this enzyme, it follows that the sulfate esters of estrone, estradiol and estriol hydrolyzed by the enzyme were conjugated at the phenolic hydroxyl at carbon 3.

37. Hormonal Factors Producing the Gametokinetic Response in the Male Frog (Rana Pipiens).

Robert B. Greenblatt, Sarah Clark (by invitation) and R. M. West (by invitation).

From the University of Georgia School of Medicine, Augusta, Georgia.

Various commercial pituitary gonadotropins, prolactin, chorionic gonadotropin, equine gonadotropin and pregnancy urine have been found to produce the gametokinetic response in the adult male frog (Rana pipiens) and the minimal dose necessary to give this response has been determined.

The minimal dose of gonadotropin necessary to produce the gametokinetic response in the Rana pipiens frog has been compared with the minimal amount of gonadotropins necessary to produce ovarian hyperemia in immature Sprague-Dawley rats. In general, smaller amounts of the gonadotropins will give a positive rat hyperemia test than are necessary for the release of sperm in the male frog (Rana pipiens).

The male frog (Rana pipiens) has proved to be a reliable test animal for the diagnosis of pregnancy. In our series of 111 cases there have been 5 false negative tests. In all cases the rat ovarian hyperemia test and the Rana pipiens test were employed. No false positive frog tests were encountered. The rat hyperemia test was positive in the 5 cases in which discrepancy was noted.

38. Action of Estrogens on Release of Luteinizing Hormone in Menopausal Women.

Arthur A. Hellbaum, J. W. Funnell (by invitation) and E. C. Keaty (by invitation).

From the University of Oklahoma School of Medicine, Oklahoma City, Oklahoma.

Changes in the amounts of luteinizing (LH) and follicle stimulating (FSH) hormones in the urine from women in the menopause were determined before, during, and after the administration of various estrogens. Ovarian changes in normal and hypophysectomized immature rats were used for the assay of the gonad stimulating factors.

With large doses of estrogenic preparations it was possible to demonstrate the presence of LH in the urine of patients in whom prior to treatment only FSH had been demonstrable. The increased presence of LH in the urine was correlated with symptomatic improvement. Estradiol esters were more effective than stilbene derivatives in releasing the LH from the pituitary.

39. The Hormonal Pattern in Pseudocyesis.

A. E. Rakoff and Paul H. Fried (by invitation).

From the Jefferson Medical College and Hospital, Philadelphia, Pa.

This study included 24 women presenting the typical clinical manifestations of pseudocyesis, including amenorrhea or hypomenorrhea, abdominal enlargement, weight gain, enlargement of breasts with galactorrhea, plus a strong belief that they were pregnant and felt fetal movement. Urine hormone assays consistently showed absent or low titers of gonadotropins and high titers of estrogens. Endometrial biopsies in 10 patients showed evidence of progesteronic activity in 9 cases, with a late secretory endometrium in

2, early secretory endometrium in 2 and a mixed type of endometrium in 5. It is postulated that these patients have a persistence of corpus luteum functions, probably maintained by luteotropic hormone (prolactin). Preliminary studies indicate the presence of the latter in the urine extracts of 4 patients, tested by the method of local application to the crop sac of the pigeon. This mechanism adequately explains most of the clinical features observed. Psychiatric interviews suggest that the alteration in endocrine function has its origin in a profound psychic disturbance.

40. Management of Threatened Abortion in the Human with Large Doses of Diethylstilbestrol.

A. B. Abarbanel.

From the Department of Obstetrics and Gynecology and the Institute of Experimental Medicine, College of Medical Evangelists, Los Angeles, California.

In a consecutive series of 100 cases of threatened abortion lactose placebos were alternated with diethylstilbestrol.

There were 50 cases of threatened abortion occurring before day 70 (from first day of last regular menses). With placebos, the salvage rate in the control group was 20%. With diethylstilbestrol the salvage rate was 35%.

In the 50 cases occurring between day 70 and 120, the salvage rate was 40% for controls and 84% for those treated with diethylstilbestrol.

It is postulated that 1) the poor salvage rate before day 70 of gestation is due to the fact that most of these cases are caused by blighted ova, or disturbances in the trophoblast, as well as a break in the vascular continuity of the endometrial vascular bed; 2) after day 70 of gestation, when organogenesis is more complete, the most common cause of threatened abortion is a break in the placental-endometrial vascular bed. Since estrogen is a prime factor in maintaining vascular continuity of the endometrial vessels, it is felt that large doses of estrogen will repair the break in the dike, so to speak, and thus the pregnancy will tend to be salvaged.

Joint Meeting with The American Diabetes Association

2:00 P.M.—C. H. BEST, PRESIDING

41. A Hyperglycemic Factor Extracted from the Pancreas.

I. J. Pincus (introduced by A. E. Rakoff).

From the Department of Physiology, Jefferson Medical College, Philadelphia, Pa.

Studies have been performed on a hyperglycemic factor obtained from the pancreas. This substance can be extracted from normal dog pancreas, from pancreas rendered fibrotic by ligation of the pancreatic ducts, pancreas obtained from animals previously treated with alloxan, and fetal calf pancreas before the acinar tissue is secretory. All brands of insulin tested have also shown the presence of this activity, best demonstrated when American insulins have been treated so as to destroy their hypoglycemic action.

Administration of this factor in dosage sufficient to elevate blood sugar levels significantly had no effect on blood pressure. It was also ineffective in stimulating the external secretory activity of the pancreas.

It is possible to demonstrate the ability of this factor, when administered intravenously, to prevent hypoglycemia produced by small doses of insulin. However, when this substance has been administered after large amounts of insulin, we have not succeeded in preventing a fatal outcome in a significant number of animals.

Further studies are being carried out on the possible physiologic significance of this substance.

42. Studies in Carbohydrate Metabolism in Decerebrate Rats.

Evelyn Anderson and Webb Haymaker (by invitation).

From the National Institute of Health, Bethesda, Md., and Army Institute of Pathology, Washington, D. C.

It has been demonstrated by Claude Bernard and by numerous other workers since his time that in acute experiments in rabbits and cats, piqûre of the floor of the fourth ventricle or decerebration at the pontile level gives rise within less than an hour to hyperglycemia and glycosuria and that the increase in the blood sugar level is usually maintained for three or four hours, but may last as long as nine hours. In all of these experiments the animals generally survived not more than a day or two.

It is the purpose of this communication to describe the effects of decerebration upon the carbohydrate metabolism of rats which survived the operation for one to three weeks. Decerebration was done at pontile and midbrain levels. Glucose tolerance tests, insulin sensitivity and liver and muscle glycogen determinations were done on the rats three to seven days after decerebration. There was a marked elevation of the glucose tolerance curve during this postoperative period. The insulin sensitivity and the levels of glycogen in liver and muscle appeared to be normal.

43. Factors Affecting the Volume of the Islands of Langerhans.

R. E. Haist, Margaret Evans and B. Kinash (introduced by C. H. Best).

Abstract not presented.

44. Studies on the Serum Potassium in Diabetic Acidosis.

Carl S. Nadler, Samuel Bellett and Mary Lanning (introduced by C. H. Best).

Abstract not presented.

45. Pyruvic and Citric Acid Metabolism.

Max Miller and Ernest Bueding (introduced by C. H. Best).

Abstract not presented.

46. Changes in Inorganic Serum Phosphorus During the Intravenous Glucose Tolerance Test as an Adjunct to the Diagnosis of Early Diabetes Mellitus.

Peter H. Forsham (by invitation), Marcel Roche (by invitation) and G. W. Thorn.

Abstract not presented.

47. The Metabolism of Glucose and Galactose when Administered Simultaneously to Man.

G. C. Walsh (by invitation), M. M. Hoffman, H. T. McAlpine (by invitation) and E. H. Mason (by invitation).

From the McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

A method which might be helpful in the elucidation of the pathogenesis of hyperglycemia in man is presented. A solution containing 25 Gm. of glucose and 25 Gm. of galactose is administered intravenously to subjects in the postabsorptive state. From specimens of venous blood the glucose and galactose concentrations at 15, 30, 45, 60 and 120 minutes following the injection are determined. The rate of removal of each sugar from the blood stream is expressed as a Removal Constant which is calculated from the concentrations of that sugar at 15 and 45 minutes. The results obtained in normal subjects and patients are shown in the following table.

Condition of Subjects	Number	Glucose Removal Constant		Galactose Removal Constant	
Normal	33	2.1	(1.0 –4.0)*	6.7	(4.5–9.9)*
Acute hepatitis	12	0.92	(0.42–2.27)	3.1	(2.3–4.0)
Chronic hepatitis	11	0.8	(0.5 –1.3)	2.4	(1.2–3.9)
Diabetes	28	0.57	(0.03–1.52)	6.0	(4.0–8.7)

* Mean and range.

These results indicate that patients with hepatitis who have impairment of glucose tolerance also have impairment of galactose tolerance. Patients with diabetes, on the other hand, show impairment only of glucose tolerance. The possible application of this procedure to the investigation of the causes of hyperglycemia will be discussed.

48. Studies in Fat Metabolism. I. Steroid Hormonal Effects Upon Blood Ketones and Other Intermediate Products of Fat and Protein Catabolism.*

Laurance W. Kinsell, Sheldon Margen** (by invitation), George D. Michaels (by invitation), Betty T. Signorotti (by invitation) and David P. McCallie (by invitation); with the technical assistance of Vernon T. Thompson, Robert V. Deal, and Philip LoDuca.

From the Division of Medicine, University of California Medical School, Metabolic Research Unit, U. S. Naval Hospital-University of California, and the Department of Medicine, U. S. Naval Hospital, Oakland and San Francisco, California.

A patient with severe diabetes mellitus complicated by severe thyrotoxicosis was followed under balance study conditions for a period of more than three months. His diet and insulin were so regulated as to cause him to catabolize more than 260 Gm. of fat daily, to lose approximately 75 Gm. of glucose in the urine each day, and to be in negative nitrogen balance. Under these conditions, his fasting blood ketones were more than 30 times the normal level and he had extensive ketonuria.

With all other factors remaining constant, testosterone propionate administered to this man in a dosage of 150 mg. daily, produced a profound protein anabolic effect, a decrease of glycosuria of proportional degree (D/N =3.65), and at the same time produced a progressive fall in the fasting blood ketones to levels approaching the normal. During this same period, his basal metabolic rate was not significantly changed, and his fat catabolism was therefore as great or greater than during the period prior to testosterone administration. The discontinuance of testosterone for a period of two days resulted in a prompt rebound in the entities above noted as well as the production of other metabolic changes which are discussed.

From the above, one must conclude that under the conditions of this study testosterone produces a profound effect upon intermediate products of fat and protein catabolism and that this effect is most strikingly manifested by a diminution in fasting blood ketones. This observation may represent an acceleration of ketolysis or a diminution in ketogenesis from protein and/or fat. The evidence in favor of each of these pos-

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** Senior Research Fellow, U. S. Public Health Service, 1947–48 and Schering Research Fellow in Endocrinology, 1948–49.

sibilities is considered, as well as the evidence which might indicate an inhibiting effect of testosterone upon the production of agents such as adrenocorticotropin, the net effect of which could be the production of excessive ketogenesis.

Additional studies in other individuals relating to the effect of testosterone upon infused sodium aceto acetate are also presented.

49. The Urinary Excretion of Corticosteroids in Diabetic Acidosis.

Janet W. McArthur, Randall G. Sprague and Harold L. Mason.

From the Massachusetts General Hospital, Boston, Mass., and the Mayo Clinic, Rochester, Minnesota.

The urinary excretion of corticosteroids has been measured chemically in 6 patients with diabetes mellitus during the first 24 hours after their admission to the hospital in diabetic acidosis, and during a 24-hour "control" period after their recovery. It was found that the rate of urinary excretion of corticosteroids during the period of acidosis was from two to eight times as rapid as during the control period. There appeared to be a direct correlation between the severity of the acidosis, as judged by the level of CO_2 combining power on admission, and the rate of excretion of corticosteroids.

The temporal relation between clinical manifestations of diabetic acidosis and laboratory indications of increased adrenal cortical activity was investigated by withdrawing insulin from a diabetic patient under controlled conditions. Adrenal hyperactivity could be detected by a fall in circulating blood eosinophils within 24 hours after the withdrawal of insulin. However, an increase in the rate of excretion of corticosteroids could not be demonstrated until mild acidosis was clinically apparent.

50. "Steroid Diabetes" Associated with Cushing's Syndrome and Excretion of 17-Hydroxycorticosterone (Compound F) in Urine; Metabolic Studies.

Randall G. Sprague, Alvin B. Hayles (by invitation), Harold L. Mason, Marschelle H. Power (by invitation) and Warren A. Bennett (by invitation).

From the Mayo Clinic, Rochester, Minnesota.

Observations were made in the case of a 14-year-old boy with severe diabetes associated with Cushing's syndrone. The urinary excretion of "11-oxysteroids" was remarkably high (about 17 mg. daily, or about 20 times normal). This finding led to the isolation of 17-hydroxycorticosterone (compound F), 191 mg. of purified hormone being obtained from a twenty-five-day collection of urine.† The diabetes exhibited the features of "steroid diabetes" in animals. It was severe, glycosuria being incompletely controlled with 130 units of insulin daily. Unlike the usual behavior in ordinary juvenile diabetes, however, the urine became virtually free of sugar (2.4 Gm. in twenty-four hours) and ketonuria was absent when food and insulin were withheld for twenty-four hours. Nitrogen balance was markedly negative with an intake of 57 Gm. of protein daily. As suggested by the presence of osteoporosis, the balances for calcium and phosphorus were also negative.

The patient died after resection of one adrenal gland. At necropsy, pronounced hypertrophy and hyperplasia of the adrenal cortices, a small thymoma, a parathyroid adenoma, osteoporosis and bilateral renal calculi were found.

The metabolic behavior outlined, plus the finding of 17-hydroxycorticosterone in the urine, seems to justify the conclusion that the patient had "steroid diabetes" analogous

† Mason, H. L. and Sprague, R. G.: Isolation of 17-hydroxycorticosterone from the urine in a case of Cushing's syndrome associated with severe diabetes mellitus, *J. Biol. Chem.* **175**: 451–456 (Aug.) 1948.

to that produced in animals by the administration of carbohydrate-active adrenal steroids.

51. Behavior of Electrolytes During Treatment of Diabetic Keto-Acidosis.

Jonas Weissberg (by invitation), Thomas H. McGavack, A. M. Shearman (by invitation) and I. J. Drekter.

From The New York Medical College, Metropolitan Hospital Research Unit, Welfare Island, New York, N. Y.

Electrolyte balance was studied in 13 diabetic patients before and after the institution of adequate insulin and fluid therapy. Specimens were collected at intervals from one to three hours for the determination of serum potassium, sodium, chloride and phosphorus, CO_2 combining power, blood sugar and urinary potassium, sodium, chloride, phosphorus and total nitrogen, before and after treatment.

All 13 patients presented a definite fall in levels for potassium in the serum at some time during the period of treatment with insulin. Five patients showed a fall in serum potassium to critically low levels. The behavior of phosphorus paralleled that of potassium; sodium and chloride tended to deviate in an opposite direction to values for potassium.

The importance of the alteration of potassium in the diabetic patient is the fact that marked lowering of the level of potassium may be associated with a definite clinical state-termed "potassium-depletion syndrome" (hypopotassemia), of which restlessness, marked muscular weakness and hypotonia, fall in blood pressure, tachycardia and occasionally diaphragmatic paralysis are the most characteristic manifestations.

Among the clinically applicable implications of the results are: 1) a means for recognizing the potassium-depletion syndrome is important to the care of all diabetics; 2) frequent determinations of potassium in the serum are necessary during the period of active treatment for the diabetic-acidotic state; and 3) potassium parenterally should be administered only when the serum level falls to critical or "near-critical" levels.

Read by Title

52. Correlation of Vaginal Smears and Endometrial Biopsies in Normal Cycles and in Gynecic Disorders.

H. E. Nieburgs, Robert B. Greenblatt and S. Bamford (by invitation).

From the University of Georgia School of Medicine, Augusta, Georgia.

In the perfunctory study of patients with gynecic disorders a discrepancy was noted not infrequently in the results of the endometrial biopsy and the vaginal cytologic smear. A close correlation should exist between the endometrial histology and the vaginal cytologic smear since both reflect ovarian function. A special study was therefore undertaken in which endometrial biopsies and vaginal smears were taken at the same time in 250 patients with various endocrine-gynecologic disorders. An evaluation of this correlative study is presented. Of particular interest was the finding of two specific types of smears: the "cytolytic" type which heretofore has been interpreted as indicative of progesterone activity and the "mucoid cornified" type which was encountered in patients with clinical evidence of increased responsiveness to intrinsic androgens. A lack of correlation with the endometrial biopsy existed in this particular group of patients. It is interesting to note that the cytolytic smear was associated in most instances with an estrogenic endometrium or cystic glandular hyperplasia, whereas the mucoid cornified type of smear appeared mainly in patients with cystic glandular hyperplasia.

53. The Effect of Pteroylglutamic Acid Antagonists on the Response of the Reproductive Accessories of C57 Male Mice to Testosterone.

E. D. Goldsmith, H. M. Black (by invitation) and R. F. Nigrelli (by invitation).

From the Department of Histology, College of Dentistry, New York University and New York Aquarium, New York Zoological Society, New York.

Preliminary investigations disclosed that the seminal vesicles and coagulating glands of castrated male mice receiving a Purina chow ration supplemented with 10 Gm./kilo of succinylsulfathiazole and a high level of a crude folic acid antagonist (x-methylpteroylglutamic acid, Lederle) responded only slightly to high dosages of testosterone.

Seventy day old mice, castrated at 50 days of age, were placed on 1) *the control ration* —Purina chow supplemented with 10 Gm. of succinylsulfathiazole per kilo, and 2) *the antagonist ration* consisting of the control ration to which a folic acid antagonist of known chemical constitution, 4-amino-N^{10}-methyl-pteroylglutamic acid (Amethopterin, Lederle) was added at levels of 17 mg. per kilo. After eleven days, the "antagonistic" animals were given daily injections of 1 mg. of testosterone propionate in sesame oil during a 5-day period. A number of "control" mice were treated similarly with the androgen, and an equal number were injected with equivalent quantities of sesame oil. All animals were sacrificed 24 hours after the fifth injection. The seminal vesicles and coagulating glands of the testosterone-treated mice on the control diet exhibited the characteristic hypertrophy resulting from androgen treatment. The sesame oil elicited no visible growth. The mice fed Amethopterin showed a reduced response to the testosterone, and differed but slightly from the control stock animals which received sesame oil. Histologic studies are reported.

54. Synthesis of Testosterone from Androstenedione-3,17 by Testis Tissue.

Leo T. Samuels, Blaine H. Levedahl (by invitation), M. L. Helmreich (by invitation) and M. M. Pottner (by invitation).

From the Department of Biochemistry, College of Medicine, University of Utah, Salt Lake City 1, Utah.

Androstenedione-3,17 has been incubated with testis slices from several species of animals. Evidence from colorimetric reactions and biologic assay indicates that this tissue is able to reduce the ketone group on carbon 17, thus forming testosterone. The metabolism of other steroids by testis tissue has also been studied in an attempt to trace a possible route of synthesis of the hormone from constituents known to be present in considerable amounts in testis tissue.

55. The Role of the Adrenal Cortex in Some Somatc-Sexual Aberrations in Infants and Children.

M. M. Melicow.

From the Department of Urology, Columbia University, College of Physicians and Surgeons, New York 32, N. Y.

The article is based on a study of somato-sexual aberrations in infants and children seen at the Squier Urological Clinic and Babies Hospital of Columbia University-Presbyterial Hospital.

In this study are presented 2 true hermaphrodites, 13 pseudohermaphrodites, and 7 children with accelerated puberty characteristic of their own or opposite sex.

It was found that at birth and in early infancy the problem is mainly one of ascertaining the true sex and correcting the deviation. Several females were brought up as males

and vice versa; in a number, the true sex was in doubt from birth. Awareness of error in some cases was not realized until puberty. At birth and in early infancy, clinical, laboratory and radiologic evidence of cortical hyperfunction is usually not demonstrable. In some it becomes manifest later. Steps necessary to demonstrate cortical hyperfunction ±neoplasia are presented. In childhood, the problem is mainly one of halting the tendency to apparent sex-reversal and removing the cause. The true sex had been ascertained at birth but, because of a profound heterosexual endocrine drive, a somatosexual aberration developed. Cortical hyperfunction was demonstrable in all cases.

56. Effect of Compound E on Blood Ketone Bodies.

Leslie L. Bennett (by invitation), Alexander Slessor (by invitation) and George W. Thorn.

From the Department of Medicine, Harvard Medical School and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Massachusetts.

Fasting (8 a.m.) blood ketone bodies were determined in 7 patients with Addison's disease before, during and after treatment with 100 mg. daily of synthetic Compound E acetate (11-dehydro-17-hydroxy corticosterone). Control levels varied from 0.34 to 1.27 mg. per cent. In 6 patients a significant rise in blood ketone level occurred following the intial 24-hour period of Compound E administration. The values varied from 1.21 to 2.57 mg. per cent, being higher in each instance than any previous control level for that patient. The values returned to, or toward, control levels during continued therapy. This was associated, however, in the one patient in whom the respiratory quotient was measured, with a persistent reduction in the fasting nonprotein respiratory quotient throughout the period of therapy.

In 4 patients blood ketone and blood sugar levels were followed during the last 12 hours of a 24-hour fast. Blood ketone and blood glucose values were maintained at a higher level during the period of Compound E administration, as compared to control values obtained with prolonged fasting in the untreated state. It is suggested that, in man, Compound E facilitates the breakdown at fat via ketone bodies.

57. The Effect of Dietary Protein on the Ability of the Liver to Inactivate Estradiol in the Rat.

Joseph W. Jailer.

From the Endocrine Section of the Medical Services, and the Chemical Laboratory of The Mount Sinai Hospital, New York.

Biskind has claimed that in vitamin B deficiency the liver loses its ability to inactivate estrogens. It had been previously shown that it was not the lack of components of the B complex per se that was responsible in this mechanism, but the concomitant inanition (Jailer).

Spayed female rats with pellets of estradiol implanted in the spleen were placed on isocaloric vitamin B deficient diets containing 5, 15, and 50% protein. After an average of twenty days, the rats on the 5 and 15% protein diets showed signs of estrus as determined by vaginal smear, while those on a 50% protein diet remained in diestrus. Uterine weights at the termination of the experiment were: 5%-99.0 mg.; 15%-128.0 mg.; and 50%-65.7 mg. Other similarly treated rats were placed on a diet which was the same except for ample vitamin B complex. The food intake however, was restricted to 3 grams per day per rat. After 25 days on this regimen, the 5 and 15% groups showed signs of estrogen activity, with average uterine weights of 121.6 and 90.0 mg. respectively, while the 50% group did not (uterine weights 67.2 mg.).

58. Pregnanediol Excretion in Cases of Blighted Ovum.

A. B. Abarbanel, Robert Hoyt (by invitation) and M. G. Levine (by invitation).

From the Institute of Experimental Medicine, College of Medical Evangelists, Los Angeles, California.

Pregnanediol excretion was determined quantitatively by a modification of the Astwood-Talbot method and was measured quantitatively spectrophotometrically.

In a series of infertility patients who conceived, pregnanediol excretion was measured at least once a week. In 4 cases in which a blighted ovum was recovered the pregnanediol excretion fell to or towards zero from a previously normal level from two to four weeks before the blighted ovum was expelled. Diagnosis was confirmed histologically. In two cases, the test for chorionic gonadotropin was found to be positive.

In 17 other cases, a presumptive diagnosis of blighted ovum was made on the basis of a drop in pregnanediol with a positive test for chorionic gonadotropin.

From these findings, it is postulated that a viable more or less normal embryo may be actively concerned with the metabolic conversion of progesterone to pregnanediol in the human.

59. A Mechanism of Potassium Deficiency in Alkalosis.

Charles H. Burnett, Belton A. Burrows (by invitation) and Robert R. Commons (by invitation).

From the Evans Memorial Hospital and the Department of Medicine, Boston University School of Medicine, Boston, Massachusetts.

Evidence of intracellular potassium deficit has been demonstrated in a series of 12 patients with alkalosis resulting primarily from loss of gastric contents; some were refractory to treatment until potassium was supplied. The mechanism is not clear, but the authors' observations indicate that renal excretion of potassium, which exceeded that anticipated from protein catabolism alone, was an important feature. At least three factors operated in this renal loss: 1) the failure of normal kidneys completely to conserve potassium, even in the presence of potassium deficit; 2) the even greater inability to conserve potassium in renal insufficiency (which has regularly occurred in this type of alkalosis); 3) increases in potassium excretion after administration of sodium salts and hence accentuation of any previous deficiency of this cation (saline was always administered in cases observed). Estimations of tubular reabsorption of various ions (calculated from inulin clearance) indicated that this last effect may be due to inhibition by sodium of renal tubular potassium reabsorption. Alternatively, since there is some evidence that potassium is actively secreted by renal tubules, sodium may stimulate potassium secretion. It is suggested that this phenomenon may offer a clue to solution of the general problem concerning the mechanism of transfers of sodium and potassium across cell membranes.

60. Effects of Hemopoietic Agents on Blood Formation in Hypophysectomized Rats.

Robert Gerstner (by invitation) and Albert S. Gordon.

From the Department of Biology, Washington Square College of Arts and Science, New York University, New York.

The anemia induced in the rat by hypophysectomy can be effectively prevented or overcome by appropriate hormonal therapy (Gordon and Charipper; Crafts). Attempts were made in the present work to influence blood formation in the hypophysectomized rat by the administration of nonendocrine hemopoietic factors. The combination of folic

acid, liver extract, iron and copper evoked marked reticulocytosis but lowered RBC counts and Hb concentrations. RBC fragility values and sedimentation rates were increased by this treatment. Protein hydrolysates caused slight reticulocytosis, decreased RBC numbers, hemoglobin concentrations and fragility values but sedimentation rates were increased. Treatment with the combination of protein hydrolysates, iron, copper, liver extract, folic acid, ascorbic acid, and certain members of the vitamin B complex induced a strongly marked and continued reticulocytosis. RBC numbers rose only slightly. Hb concentrations and RBC fragility values were not altered. RBC sedimentation rates were greatly increased. The results of additional experiments now being conducted with santhopterin and vitamin B_{12}, singly and in combination with other factors, are also reported.

61. The Relative Effectiveness of Desoxycorticosterone Acetate in Oil Solution and in Pellets Diluted with Cholesterol.

Albert Segaloff.

From the Department of Medicine, Tulane University and The Alton Ochsner Medical Foundation of New Orleans, Louisiana.

Groups of 15 to 20 immature male Fisher rats weighing 40 to 60 Gm. were adrenalectomized. At operation either a pellet was implanted or the first injection of desoxycorticosterone acetate (DCA) in sesame oil was made. The pellets were removed, or injections stopped after 28 days.

Twenty controls with pellets containing only cholesterol and 20 controls injected with sesame oil died on the fourth, fifth or sixth day after bilateral adrenalectomy.

Pellets with 100%, 75% and 50% DCA produced essentially normal growth. Animals implanted with 5% DCA pellets all failed to survive the 28-day experimental period. Growth with pellets of intermediate percentages of DCA was proportionate to the percentage of DCA. Of the animals injected with DCA, none receiving 10γ per day survived the 28-day experimental period. Those receiving 125γ daily grew at essentially the normal rate. The intermediate levels produced growth curves differing in proportion to the amount of DCA given.

Comparing the curves reveals that growth was essentially the same for a given amount of DCA either in terms of absorption or injection.

62. Adrenal Cortex Activity in Essential Hypertension.

Louis Tobian, Jr. and Harold Joseph (introduced by Carl A. Bunde).

From the Southwestern Medical College, Dallas, Texas.

We have previously shown that the urinary "formaldehydrogenic" corticosteroid excretion was normal in essential hypertension.

Four other procedures have been carried out to examine further adrenal cortical activity in hypertensive patients.

I. Plasma corticosteroids:

The purified lipid fraction was analyzed by both the Heard-Sobel and Corcoran-Page-Lowenstein methods for corticosteroids. The 8 hypertensive patients and 7 normotensive patients exhibited similar plasma "corticosteroid" levels.

II. Fasting blood eosinophil concentration:

Average of 46 normotensive patients: 139.9/cu. mm. (S.D. = $\pm$85.6).

Average of 40 essential hypertensive patients: 107.7/cu.mm. (S.D. = $\pm$77.7).

probability = .07

III. Plasma CO_2 combining power:
Average of 11 normotensive patients: 31.1 mEq./L. Average of 19 severe hypertensive patients: 33.2 mEq./L.
probability = .6.

Even though 42 per cent of the hypertensive patients had a value greater than any one in the normotensive group, the differences observed were not significant.

IV. Plasma CO_2/Cl ratios:
Average in 11 normotensive patients: .307.
Average in 19 severe hypertensive patients: .335.
probability = .4.

Even though 32 per cent of the hypertensive group had ratios higher than any one in the normotensive group, the difference in the group were not significant.

Although it is possible that there might be a slight adrenal hypersecretion in essential hypertension, the above data indicate that a marked adrenal hypersecretion is unlikely.

63. The White Blood Cell Response of Rats to Adrenalectomy, Stress, and Pantothenic Acid.*

Mary E. Dumm, Paul Roth (by invitation), Paul Ovando (by invitation) and Elaine P. Ralli.

From the Laboratories of the Department of Medicine, New York University College of Medicine, New York.

Large doses of calcium pantothenate have been shown to modify the response of the white blood cells of the rat to adrenalectomy and, in intact rats, to stress. The total white blood cells and lymphocytes were counted in tail blood from young rats kept for thirty days on a pantothenate deficient diet. Following adrenalectomy, the total white blood cells and lymphocytes were followed for twenty days in groups of rats receiving from 0 to 4 mg. of pantothenate daily. Large doses of calcium pantothenate delayed and partially suppressed the increase in total white blood cells and lymphocytes which usually follows adrenalectomy. In another series of experiments the total white blood cells and lymphocytes were counted before and at intervals after moderate stress (swimming) in both intact and adrenalectomized rats on diets providing various intakes of pantothenate. No consistent changes in white blood cells were found after stress in any group of adrenalectomized rats. Intact rats on the high (4 mg. per day) pantothenate diet showed a delayed lymphocyte response to stress as compared with rats on a moderate (0.1 mg.) pantothenate intake. The low initial lymphocyte values previously reported in intact pantothenate deficient rats were further depressed following stress.

64. Role of Emotional Stress in the Survival of Adrenalectomized Rats Given Replacement Therapy.

Miguel R. Covian (introduced by Curt Richter).

From the Psychobiological Laboratory, Johns Hopkins Hospital, Baltimore 4, Md.

Previous work done in this laboratory showed that laboratory Norway rats can be adrenalectomized and then maintained almost indefinitely merely by increasing the sodium chloride available to them, but that their adrenalectomized wild Norway counterparts do not survive on salt alone after adrenalectomy, nor are cortical extracts entirely successful in keeping these wild rats alive. In an attempt to find the explanation for this

* This research was aided by a grant from the National Vitamin Foundation.

difference, 21 wild Norway rats (freshly trapped in the alleys of Baltimore), 13 Alexandrine rats and 23 laboratory rats on a high salt diet were adrenalectomized and had percorten pellets implanted between the shoulder blades. Twenty days after the operation the rats were replaced in pairs in a box with a floor made of metal rods and subjected to repeated electrical shocks during a period of five minutes. This treatment caused the wild rats (both Norway and Alexandrine) to fight each other with great intensity, but the laboratory rats merely sought to escape, jumping and squealing, and only occasionally struck out against each other. The wild rats which were not operated on fought violently but did not die.

The mortality resulting from this treatment was much lower for the laboratory rats than for the wild ones, indicating that the emotional state of captive wild rats is such that therapy sufficient to offset the loss of adrenals in laboratory rats is insufficient to keep the wild rats alive.

65. The Androgenic Activity of New Esters of Testosterone.

A. J. Bergmann and Lloyd C. Miller (introduced by John S. L. Browne).

From the Sterling-Winthrop Research Institute, Rensselaer, New York.

Numerous new esters of testosterone have been assayed on castrated rats using testosterone propionate as the reference standard. The acids from which the esters were formed included alkoxy- and alkylmercaptoalkanoicacids in which the alkanoic chain contained 2, 3 or 4 carbon atoms. It was found possible to classify the esters into three groups according to their androgenic activity relative to testosterone propionate. Most of the esters were distinctly less active than the latter, or just equalled it, but six were significantly more potent. Two of this latter group, testosterone ethylmercaptoacetate and testosterone methylmercaptoacetate are especially potent, being 123 and 133% as strong as the propionate when compared on the basis of molar equivalents of testosterone. Assayed by the comb-growth response induced in three-day old chicks, comparable potencies were observed. These esters have been studied extensively enough to justify clinical trial.

66. Effect of Androgen and Growth Hormone on the Rat's Os Penis.

Wm. R. Lyons, Edward Abernathy (by invitation) and Mark Gropper (by invitation).

From the Division of Anatomy and Institute of Experimental Biology, University of California, Berkeley, California.

Rats hypophysectomized and castrated at the age of 26 days show almost complete growth stasis and their accessory sexual structures remain infantile. The proximal cartilaginous cap of the os penis becomes atrophic.

Testosterone propionate (0.1 mg. daily subcutaneously for 21 days from the day of operation, promotes a gain of approximately 1 gram daily in such animals. The penis shows preputial splitting within seven days and grows at a faster rate than in normal controls. Although general skeletal growth is retarded as in uninjected controls which underwent operation, the penile bone continues to grow as in normal rats. Cartilaginous condyles develop proximally and following their proliferation they are replaced by apparently normal ossification.

Pure hypophyseal somatotropin (Li and Evans) similarly administered in daily doses of 1 mg. induces a weight increase of skeletal growth equalling that of normal animals; but the accessory sexual organs including the penis and its ossicle remain atrophic.

A combination of the above mentioned doses of somatotropic and androgenic hormones causes a daily body weight gain of almost one gram more than that induced by somatotropin alone; and the growth response of the os penis was slightly greater than to androgen alone.

67. The "Thiocyanate Space" and "Iodide Space" in the Thyroid Gland.

J. F. McClendon, William C. Foster (by invitation) and Emerson Reed (by invitation).

From the Department of Physiology and Research Laboratory of Physiology, Hahnemann Medical College, Philadelphia.

In the study of thiocyanate goiter, errors arise in the determination of the "thiocyanate space" and the "iodide space" in the thyroid gland due to formation of ferrous thiocyanate and to enzyme action during the extraction. To avoid the former, the concentration of ferric ions was increased 10 times and the spectrophotometer was used at 5000°A. To avoid the latter the thyroid was frozen, quickly sliced with a razor, crushed in a supercooled diamond mortar and quickly homogenized in a Wisconsin homogenizer. The proteins were dissolved in water and precipitated with trichloracetic acid. The "iodide space" is larger than the "thiocyanate space." Thiocyanate cannot interfere with the uptake of iodide by the thyroid unless it is in relatively high concentration.

68. A Comparison of the 17-Ketosteroid Excretion of Cases of Cushing's Syndrome Due to Adrenal Tumor With Those Due to Hyperplasia (Hyperfunction).

Anne P. Forbes, Evelyn L. Carroll (by invitation) and Mary L. Wheeler (by invitation).

From the Massachusetts General Hospital, Boston, Mass.

It is recognized that Cushing's syndrome may result from a benign or malignant tumor or from hyperplasia (hyperfunction) of both adrenals. Hyperplasia presumably results from an over-production of pituitary adrenocorticotropic hormone (ACTH). Since ACTH has been shown to increase the excretion of 17-ketosteroids, their excretion in such cases should be elevated.

Small adenomas may be found in hyperplastic adrenals; however, in cases where one large adenoma is found the rest of the adrenal tissue is usually atrophied. The production of ACTH has presumably been suppressed by hormones from the tumor. Urinary 17-ketosteroids should be low unless the tumor itself produces 17-ketosteroid precursors.

With malignant tumors one would expect inconstant results.

The adrenals have been visualized in 16 cases of Cushing's syndrome at the Massachusetts General Hospital. In 5 cases of benign adenoma the 17-ketosteroid excretion was low (average 4.8 mg. per 24 hours). In 9 cases of hyperplasia it was elevated (average 26 mg. per 24 hours). In 2 cases of carcinoma it was very high.

The findings in cases collected from the literature are in general agreement with the above.

69. Sex Hormones and Staphylococcus Infections.

Manuel Villaverde.

Linea 755—Vedado, Habana, Cuba.

According to the author's experience, sex hormones do not seem to act directly upon staphylococcus infections, but upon the tissue condition itself *i. e.*, they do not act like antiseptics or antibiotics, but like tissue regulators.

Two cases are recorded. The first one proved that testosterone is a substance capable of inducing furunculosis. Boils appeared following a course of 25 mg. of the hormone, thrice a week. This man developed furunculosis on three occasions following courses of testosterone, and once it was so intense as to combine several furuncles into a carbuncle. He had not had acne in his youth; and after the withdrawal of testosterone only occasional and mild furunculosis developed.

The second patient, a woman, showed a very intense axillary adenitis, which broke and discharged pus every month before the menses. This condition lasted for twelve or fourteen months, becoming worse as time elapsed. The use of 20 mg. of progesterone daily, the days before the expected menses, improved her state: only a small ulcer appeared, with little exudation. During the next intermenstrual period testosterone (12.5 and 25 mg.) was given the first two weeks, and progesterone (20 mg.) the rest of the time; and since then (after seven months) no axillary swellings were produced. But, paradoxically, an annoying acne developed, after testosterone treatment.

Brief considerations on chronic cystic mastitis and on the relationship between infections and endocrines are discussed.

70. The Problem of Allergy to Steroid Hormones.

George P. Heckel.

From the Department of Obstetrics and Gynecology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Observations of Zondek and Bromberg (*J. Obst. & Gynec. Brit. Emp.* **54**: 1, 1947) indicate that allergy to steroid hormones may be responsible for certain disorders related to the genital functions. To explore this possibility skin tests with crystalline steroid hormones have been given to 137 patients. Injections of .05 cc. of oil containing .05 mg. of the steroid were given subcutaneously (just under the corium) on the arms. Estradiol, estrone, estriol, desoxycorticosterone, pregnandiol, pregnanolone, progesterone and testosterone were used. Reactions were read in twenty-four hours, positive ones consisting of faint erythema and/or slight induration and tenderness.

Sesame oil was used in all cases and in a number the same hormones were given in both sesame and peanut oil. Many reactions were observed with sesame oil as the vehicle, but relatively few with peanut oil. The controls (.05 cc. of oil alone) remained negative. Many patients were tested more than once and sensitivity of the skin was found to be variable in the same individuals. Hyposensitization was tried in 41 cases. The highest dilution of the hormone in .05 cc. of oil which failed to elicit a reaction was given subcutaneously one to four times weekly. Twenty-two of the 41 patients improved. Symptoms related to the menstrual cycle and climacteric which were relieved included headache, nausea, dizziness, fatigue, depression, ovarian pain and mastodynia.

71. Intravenous Estrogen in Menometrorrhagia in the Human.

A. R. Abarbanel.

From the Department of Obstetrics and Gynecology and Institute of Experimental Medicine, College of Medical Evangelists, Los Angeles, California.

Mixed conjugated estrogens from pregnant mare's urine was especially prepared for intravenous use in the human. On the rationale that if the level of estrogen could be raised rapidly to a sufficiently high level, functional uterine bleeding could be easily controlled, varying doses of estrogen were used. It was found that a dose of 10 mg. was sufficient to control functional bleeding in 6–12 hours in most cases.

The various conditions under which intravenous estrogen was used also included myomata uteri, fibrosis uteri, endometrial polyps, cystic ovaries, irregular shedding of the endometrium, estrogen-deprivation bleeding. In 30 cases, a successful result was obtained in 26, or 87%.

In addition, the "stoss" effect of intravenous estrogen has been utilized in threatened abortion, severe menopausal syndrome, infertility in female associated with scanty cervical mucus, and several other conditions.

72. The Incidence of Cancer in Endocrine Case Histories.

J. K. Fancher and Jean Brooks (by invitation).

Atlanta, Georgia—478 Peachtree Street, N.E.

One thousand endocrine case records were studied and compared with the histories of 1000 cancer patients and 1000 patients without glandular disorder or cancer. These 3000 cases were taken from the files of the Good Samaritan Endocrine Clinic and the Steiner Cancer Clinic of Atlanta. There was a noticeable parallel between the incidence of cancer in the family histories of the endocrine and cancer groups, with a perceptible decrease in the negative group, *i.e.*, endocrine 29.8%, cancer 29.1%, neg. 21.5%. Malignancy was most frequent in the first and second generations of antecedents in cancer histories, whereas it was highest in the second and third generations of endocrine patients. The spread in negative cases was not remarkable. Females, exceeded males in all groupings. The G.U. and G.I. tracts were most frequently involved. Various charts are shown. All histories were carefully checked and only complete ones used. Although a factor of error in such statistical material cannot be dismissed, it is thought that the prevalence of cancer and the widespread use of various hormonal products enhances the importance of such a study.

73. Renal Clearances in Patients with Cirrhosis of the Liver, With and Without Ascites.*

Stephen H. Leslie, Barbara Johnson (by invitation) and Elaine P. Ralli.

From the Laboratories of the Department of Medicine, New York University College of Medicine, New York.

Twenty renal functional measurements done on 11 patients with cirrhosis of the liver, during and in the absence of ascites, included effective renal plasma flow (Cpah), glomerular filtration rate (Cln), and maximal tubular excretory capacity (Tm pah). Eight measurements were done while ascites was present, 6 when no evidence of ascites was present, and 6 were done immediately following the ingestion of 1500 ml. of tap water. In the group with ascites, 5 patients were actively reaccumulating fluid and 2 were undergoing spontaneous diuresis at the time of measurement. The average Cpah in the former group was 371 cc./min., the average Cln 69 cc./min., and the average Tm pah 62 cc./min. The averages in the latter group were: Cpah 704 cc./min., Cln 136 cc./min., and Tm pah 54 cc./min. Administration of water preceding measurements to one of the patients caused no change. In the group without clinical evidence of ascites, the average measurements were Cpah 846 cc./min., Cln 201 cc./min., and Tm pah 84 cc./min. In 4 patients measurements following a water load were: Cpah 643 cc./min., Cln 153 cc./min., and Tm pah 74 cc./min.

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* This research was aided by a grant from the U. S. Public Health Service.

Cirrhosis of the liver with actively reaccumulating ascites depressed all of the functions measured. Once diuresis began the functions measured returned to normal. When ascites had been effectively controlled, the measurements showed an elevation of renal plasma flow and glomerular filtration rate above the range of normal. Tm pah remained normal.

74. Porphyria Simulating Anorexia Nervosa.

Bernard A. Watson.

From Clifton Springs Sanitarium and Clinic, Clifton Springs, N. Y.

This case is presented because a diagnosis of anorexia nervosa was made on a patient who, for a number of years exhibited signs and symptoms of porphyria which were not recognized. The patient had progressive fatigue, intermittent moderate to severe abdominal and leg pains, nausea, vomiting, and finally a reluctance to eat. He was 6′3″ tall and weighed 87 pounds on admission to the hospital. A résumé of his signs and symptoms over a 6-year period are presented.

Of special interest is the fact that he had an appendectomy because of abdominal pain, and later a nephrectomy because of "bloody urine." His gradual withdrawal from society, his many psychosomatic complaints, together with his reluctance to eat because he felt that most foods caused his abdominal pain, resulted in such a marked state of undernutrition that a diagnosis of anorexia nervosa was made.

A routine blood count, 17-ketosteroids and albumin-globulin ratio were within normal limits. The B.M.R. was minus 14. The urine was a "bloody color" showing marked porphyria.

The cause of porphyria in this case, as well as its management, are discussed. The fact is stressed that before a diagnosis of anorexia nervosa or pituitary cachexia is made, porphyria should be considered. It is relatively easy to diagnose if borne in mind.

75. Hemosedimentation Test in Obesity.

Aulo Pinto Viégas.

Belo Horizonte, Minas, Brazil.

Two hundred and twenty-six cases of obesity (obesity is defined as increase of at least 20% in weight), 179 women and 47 men, from 5 to 65 years of age, are presented. Their checkup included: clinical examination, blood studies, roentgenogram of skull and chest radioscopy. A classification has been made according to the degree of overweight:

		Hemosedimentation	
Number of cases	Overweight percentage	20 mm. and up	Average of each group
1st group 48	1 to 10	16 cases or 33.3%	18.0 mm.
2nd group 68	11 to 20	23 cases or 34.7%	18.2 mm.
3rd group 49	21 to 30	16 cases or 33.4%	18.2 mm.
4th group 29	31 to 40	16 cases or 69.4%	26.2 mm.
5th group 22	41 to 50	15 cases or 68.0%	30.4 mm.
6th group 10	51 and up	3 cases or 30.0%	17.0 mm.

Roughly ⅓ of all cases had a hemosedimentation rate over 20 mm. (1 hour, Westergreen), without a close relationship between the degree of obesity and this test. The presence of edema, of intestinal parasitosis and the classification of various kinds of obesity do not throw any light on the problem. The obese should be investigated more thoroughly, as they are more liable to premature death than normal people.

76. The Effects of Vitamin B, Thyroid, and Adrenal Alterations on the Amino Acid Oxidase Activity of Rat Liver and Kidney.

Samuel R. Tipton and Frances M. Colvin (by invitation).

From the Department of Zoology and Entomology, The University of Tennessee, Knoxville.

Albino rats were maintained on diets with deficiencies in all or some of the B vitamins. The *d*-amino acid oxidase of liver and kidney tissue is decreased (at least in activity) in total B deficiency and in riboflavin deficiency but is not affected significantly by thiamin deficiency. Administration of desiccated thyroid powder to animals on normal diet increases the enzyme activity. The increase is less in conditions of B deficiency and may not appear at all in severe riboflavin deficiency when thyroid powder or thyroxine is given. After adrenalectomy the enzyme activity is decreased to a small extent. The decrease is aggravated by all vitamin B deficiency conditions that have been studied. A protein-deficient diet results in a decrease in the oxidase activity but the effect is much less than that resulting from riboflavin deficiency.

77. The Problem of Endemic Goiter in Yunnan Province.

Isidor Greenwald.

From the Department of Chemistry, New York University College of Medicine, New York.

In a recent review (Curtis, G. M. and M. B. Feitman: *J.A.M.A.* **139**: 28, 1949), a paper bearing the title given above (Robertson, R. C.: *J. Clin. Endocrinol.* **1**: 285, 1941) is cited as authority for the statement "This remarkable incidence of goiter in Yunnan, resultant to the military stranglehold on the areas furnishing the only supply of salt with a high iodine content, is no mere coincidence." The "military stranglehold" is the Japanese occupation of the coastal provinces. Actually, Robertson did *not* claim that goiter in Yunnan was of recent origin and as a matter of fact, it has been common in Yunnan since before 1867 (Thorel, C.: Note medicales du Voyage d'exploration du Mekong et de Cochinchine, Paris theses, 1870). Moreover, the available analyses show that the salt from the coastal provinces was not rich, but rather poor, in iodine (Wang, H., and F. W. Cheng: *Jour. Chinese Chem. Soc.* **3**: 345, 1935). As McClendon wrote, "sea salt may not be considered an adequate source of iodine," (Iodine and the Incidence of Goiter, Minneapolis, 1939). It is possible that goiter was more prevalent in 1940 than in 1867 but it is evident that the salt supply had nothing to do with its spread.

Association for the Study of Internal Secretions

Recipients of Awards for 1949

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

Doctor Ernest M. Brown, Jr. was named to receive the Ayerst, McKenna and Harrison Fellowship for 1949. He was born in 1919 and received the degrees of Bachelor of Arts from West Virginia University in 1941 and of Doctor of Medicine from the University of Pennsylvania in 1944. He served as intern and junior resident at the University of Pennsylvania Hospital 1944–46. Since that time, until April 1948, he was a member of the Army Medical Corps. Doctor Brown will work at the George S. Cox Medical Research Institute with Doctor F. D. W. Lukens on lesions of the islands of Langerhans produced by intravascular infusion of glucose, continuing his studies begun under this Fellowship in 1948.

THE CIBA AWARD

The Ciba Award for 1949 was given to *Doctor George Sayers*. Doctor Sayers developed a new and sensitive method for the assay of the adrenocorticotropic hormone of the anterior pituitary gland. He found that the ascorbic acid and cholesterol content of the adrenal glands varies inversely with the amount of ACTH administered to the test animal. Having established the method, he applied it to the problem of pituitary-adrenal relationships. The interrelation of the pituitary and the adrenal cortex, and the response of this hormonal system to a variety of stimuli are better understood and can be better studied as a result of his investigations.

Doctor Sayers was born in 1914. He received the degree of M.S. in physics from the University of Michigan in 1936 and the Ph.D. degree in physiological chemistry from Yale University in 1943. From 1943 to 1945 he served with the Office of Scientific Research and Development at Yale University and in 1945 became Assistant Professor of Pharmacology at the University of Utah.

THE SCHERING FELLOWSHIP

The Schering Fellowship for 1949 was given to *Doctor D. Lawrence Wilson*. Doctor Wilson was selected as the first recipient of this Fellowship. He will work under the direction of Doctor George Thorn in the field of metabolic and endocrine diseases.

Doctor Wilson received the degree of Doctor of Medicine at Queen's University in 1944. Following his internship he served for eighteen months as Regimental Medical Officer in the R.C.A.M.C. He then received a Medical Research Fellowship for two years from the National Research Council of Canada, working during this period in the Department of Biochemistry of the University of Toronto. He fulfilled, as Research Fellow, the requirements for the degree of Master of Arts which he received in June 1948.

THE SQUIBB AWARD

The Squibb Award for 1949 was given to *Doctor Herbert M. Evans*. About thirty years ago Professor Evans began his studies on the physiology of reproduction. Some of this earlier work was published with J. A. Long in a monograph entitled "Oestrous Cycle of the Rat," a contribution which was of great importance in the investigation and subsequent isolation of one of the ovarian hormones. During the following three decades many significant contributions to endocrinology were made by Evans and his associates, the most important perhaps being the discovery of the growth hormone and its subsequent purification and isolation.

Other important aspects of endocrinology have been studied by Evans and his associates in the University of California: 1) the corticotropic hormones of the hypophysis; 2) the gonadotropic hormones of the hypophysis, of pregnant mare serum, and of the chorion; 3) the impairment of the vaginal cycle due to vitamin A deficiency; 4) the recognition of the importance in the rat of a nutritional factor, X, for pregnancy: and subsequently, the isolation and characterization of this factor, vitamin E.

Doctor Evans was born in California in 1882. He studied medicine at Johns Hopkins, receiving the M.D. degree in 1908. He remained on the faculty of that institution until 1915 when he was called to the Chair of Anatomy at the University of California. In 1930 he was made Herzstein Professor of Biology and Director of the Institute of Experimental Biology.

American Goiter Association

RECIPIENT OF VAN METER PRIZE AWARD FOR 1949

The Van Meter Prize for 1949 was awarded to *Dr. William McKendree Jefferies* of the Massachusetts General Hospital, Boston, Massachusetts, for his essay on "Studies of the Relationship of the Thyrotropic, Exophthalmic and Fat-Mobilizing Principles of Pituitary Extract."

Dr. Jefferies was born in 1915 in Richmond, Virginia. He received the B. A. degree summa cum laude from Hampdon-Sydney College in 1935 and the M. D. degree from the University of Virginia in 1940. He served as an intern at the Massachusetts General Hospital, 1940–42. He then entered the U. S. Army Medical Corps, went overseas as a Flight Surgeon in the India-China Division of the Air Transport Command in 1943 and was discharged in February 1946 as a Lt. Colonel. From 1946 to the present he has been a clinical and research fellow in medicine at the Massachusetts General Hospital, first in the Thyroid Clinic and then in the Endocrine Clinic. During this period he was the recipient of a special fellowship fund of the American College of Physicians and of an American Cancer Society fellowship granted by the Committee on Growth of the National Research Council. After July 1, 1949 he will be at Western Reserve University Medical School, Cleveland, Ohio.

FIRST HONORABLE MENTION

First Honorable Mention was given to *Dr. Malcolm M. Stanley* of the Joseph H. Pratt Diagnostic Hospital, Boston, Massachusetts, for his essay on "The Direct Estimation of the Rate of Thyroid Hormone Formation in Man. The Effect of the Iodide Ion on Thyroid Iodine Utilization."

Dr. Stanley was born in 1916 in Henderson, Kentucky. He received the A. B. degree from Centre College of Kentucky in 1937 and the M. D. degree from the University of Louisville (Ky.) School of Medicine in 1941. After serving as interne and assistant resident at the Gallinger Municipal Hospital, Washington, D. C., 1941–43; as assistant and then chief resident in medicine at the Evans Memorial Hospital, Boston, and as assistant and then instructor in medicine at Boston University School of Medicine, 1943–46, he went to Joseph H. Pratt Diagnostic Hospital, Boston. During 1946–48 he was the recipient of a fellowship of the American Cancer Society sponsored by the Committee on Growth of the National Research Council, was a research associate in endocrinology, 1948–49, and became a staff member (gastro-enterology) in 1949. At Tufts College Medical

School, Boston, he has been an assistant in medicine, 1946–48, and an instructor from 1948 to the present time.

SECOND HONORABLE MENTION

Second Honorable Mention was given to *Dr. Ruth Cortell* of Mt. Sinai Hospital, New York, for her essay on "The Antithyroxine Activity of Thyroxine Analogs."

Dr. Cortell received the B. A. degree from Wellesley College in 1935, the Ph. D. degree in Physiology from the University of Chicago in 1939 and the M. D. degree from Yale University School of Medicine in 1948. She did research work at the University of Illinois School of Medicine, 1939–41, at the Abbott Laboratories, 1941–42, in the Thyroid Clinic of the Massachusetts General Hospital, Boston, 1942–44, and then entered Yale Medical School, graduating in 1948. During this entire period she was the recipient of the Smith, Kline & French Fellowship in Pharmacology. She served as an intern in the Mt. Sinai Hospital, 1948–49 and after July 1, 1949 will be at the Montefiore Hospital, New York.

FELLOWSHIPS FOR LATIN-AMERICAN PHYSICIANS

The American College of Physicians and the W. K. Kellogg Foundation, with the cooperation of the U. S. Department of State and of medical schools in the U. S. A., Canada and Latin-American countries, will shortly inaugurate a program of postgraduate medical fellowships. Outstanding young physicians will be nominated to the College and Foundation by local committees in the countries to the south, and those to whom fellowships are awarded will be brought to this country for a year or more of special training. It is anticipated that the first fellows will begin their studies during the autumn of 1949.

Eligibility requirements include citizenship in the country from which application is made and familiarity with its culture and economy, graduation from an acceptable medical school and completion thereafter of an internship of twelve months or more, ability to use the English language, and assurance of a subsequent teaching affiliation with a medical school in the native country. Those needing some training in English will be assigned to a special course for the purpose in the United States.

Designed to stimulate progress in the teaching of internal medicine and research, and to help the most promising young doctors of medicine in these countries to prepare for teaching and research careers in their native countries, the program also will serve to increase understanding among the American republics by serving as a medium for the exchange of knowledge and acquaintanceships.

The American College of Physicians, operating through its Committee on Fellowships and Awards, will undertake to arrange a suitable program of study in internal medicine or its subspecialities, such as cardiology, gastro-enterology, etc., in widely recognized medical education centers in this country, and to place the fellows appropriately under preceptors in these institutions.

The W. K. Kellogg Foundation will provide each fellow with a monthly stipend adequate for his basic living costs, an allowance for necessary travel within this country or Canada, and will defray the tuition for courses recommended by the preceptor and approved by the sponsors. In view of the pressing need of Latin-American medical libraries, the Foundation will reimburse the fellow for the cost of required textbooks on condition that they become the property of the medical school in which the fellow will teach upon his return home.

Representatives of the Foundation will visit the fellows periodically

during their stay in this country for conferences with them and their preceptors, thus to follow their progress. They will also be visited at intervals after their return to their home institutions in an effort to evaluate the end results of their training and to offer any possible assistance to improve teaching, research and practice in the field of internal medicine in their respective countries.